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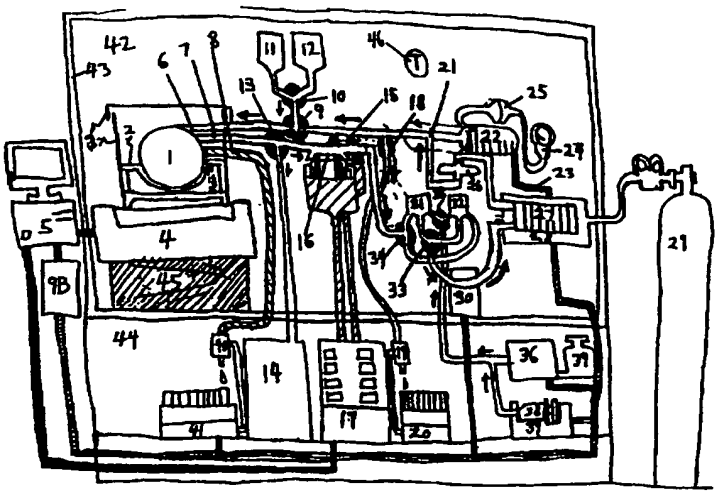

(57) Abstract

A computer-based organ perfusion apparatus is described which is capable of perfusing organs (1) at near-normal temperatures with blood or other oxygen-carrying substances. The apparatus permits organ viability to be evaluated by on-line measurements of physiological performance. Embodiments of the apparatus use a computer-driven blood pump (30) to allow physiological perfusion conditions to be established. The apparatus includes provisions (48) for the replacement of lost circulating volume and for the infusion of nutrients, drugs, and altered perfusates to assist in maintenance or recovery of organs. The apparatus may further sense perfusate gas tensions, pH, and temperature without electrical cross-interference (15 and 16), automatically measure production rates of urine, bile, pancreatic duct secretions, or other physiological exudations (8), and determine organ blood or perfusate flow rates, vascular resistance, and organ edema (4).

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ORGAN EVALUATION AND RESUSCITATION DEVICE AND METHOD

BACKGROUND OF THE INVENTION

The invention relates to a method and apparatus for perfusing an organ. The perfusion of the organ permits the viability of the organ to be maintained and evaluated. Further, the perfusion of the organ by certain perfusates or fluids, including blood, can resuscitate the organ to a point where it is viable.

The shortage of donor organs for transplantation has been a long-term problem which has seen very little improvement over the past 10 years. There are very few donors with organs that are viable and able to be effectively transplanted. It has been estimated that the number of potential donors would double if it were possible to use organs after the cardiac arrest of potential donors. This could increase the net number of available organs from the current 5,000 donors or so to about 10,000 donors per year in the U.S., with the addition of perhaps another 5,000 donors per year outside of the U.S.

Certain organs are known to be viable after as long as 30 to 60 minutes or more of warm ischemia, based on animal studies. In these studies, the flow of fluid to individual organs was selectively interrupted and then re-established. However, when flow to the organ is interrupted by irreversible cardiac arrest or blood loss of a potential organ donor, organ resuscitation is more problematic. A machine into which an organ can be placed to permit the organ to be reperfused under controlled conditions is needed. A machine of this type would terminate the ischemic insult and allow the organ to begin to self-repair.

The device would also be useful if a potential donor has suffered cardiac arrest. The device can be used between cardiac arrest and organ excision for perfusion in

vitro, to flush donor organs in vivo and allow deterioration to be arrested and recovery to be initiated even before the organ is able to receive the benefit of perfusion in vitro.

A variety of pharmacological agents, such as ATP-$MgCl_2$, have been shown to be effective in the treatment of organ ischemia. These agents permit organ recovery after the ischemic period, but most of these agents are difficult to use in vivo due to their tendency to produce hypotension. Further, the deteriorated condition of the donor may not support circulation and perfusion for a sufficient period.

The possibility of resuscitating damaged organs by normothermic blood perfusion has been demonstrated by Rijkmans et al., 37 Transplantation, 130-134 (1984). Rijkmans found that 3 days of cold storage injury could be reversed by a period of normothermic blood perfusion on a heart lung machine. This demonstration was not pursued by other workers, perhaps because a heart lung machine is not a practical device for this type of intervention.

A practical working device would preferably include a disposable perfusion circuit that could be easily replaced and a compact unit that requires a relatively low perfusate volume. This is in direct contrast to a heart lung machine, which is designed to support an entire human patient. A perfusion device containing a disposable circuit with a minimal perfusate volume has not been previously described.

A computerized control and data tracking makes organ regeneration sufficiently convenient to be practical. Although U.S. Patent No. 5,217,860 to Fahy et al. and Fahy, 28 Biomed. Instrum. Technol., 87-100 (1994), Adem et al., 3 J. Biomed. Eng., 134-139 (1981), U.S. Patent No. 5,338,662 to Sadri, and U.S. Patent No. 5,051,352 to Martindale et al. have described computer-based perfusion machines, none of the circuits in these machines combines a computer with a disposable circuit. Further, none of these circuits emphasizes a minimal

perfusate volume. None has described a disposable circuit, provisions for maintaining perfusion for lengthy periods by permitting replacement of lost fluid volume and of metabolized nutrients, or provisions for changing perfusate composition either gradually or abruptly, all of which are features that may be required for optimal organ resuscitation. In addition, the Sadri and the Martindale devices are directed toward perfusion of hearts, and Rijkmans' work emphasizes the importance of normothermic perfusion of kidneys, whereas equipment is needed that can be used to perfuse any organ.

Pacini et al., 56 Boll. Soc. Ital. Biol. Spec., 2497-2503 (1980) and 6 Renal Physiol., 72-79 (1983) showed that rabbit kidneys could be maintained in a good functioning state for over an hour during normothermic blood perfusion. This perfusion required the removal of leukocytes and platelets from the blood prior to use. Perfusion under such conditions might allow damaged organs to recover prior to subsequent cooling and storage or transplantation. Similarly, oxygen-carrying compounds other than blood may maintain and restore function near normothermia. However, Pacini did not describe useful apparatus for resuscitating organs nor the use of a computer.

Another desirable feature for the organ transplantation field is a means which can assess the viability of an organ prior to transplantation. Recently, claims have been made that organ vascular resistance provides an adequate test of organ viability. These claims must be evaluated in the light of a vast amount of literature which states the contrary.

Fahy et al., 31 Cryobiology, 573 (1994) reported that the viability of rabbit kidneys after former cryoprotectant perfusion was predicted by blood reflow rates manually measured in the first 45 minutes of blood reflow. However, quantifying vascular resistance by manual means is not practical for routine organ processing. Khirabadi and Fahy, 31 Cryobiology, 10-25

(1994) showed that the ability of a computer to collect immense amounts of data concerning flow rate and organ temperature permitted observation of a relationship between flow rate and organ temperature that would otherwise not have been detected.

The best method known so far for assessment of organ viability is in-vitro perfusion with a corresponding functional performance assessment. However, no commercially available apparatus exists to permit this assessment.

Furthermore, no previously known perfusion device contains provisions for urine (or bile or pancreatic duct fluid or other exuded fluid) collection and replacement of lost volume. None of the above-described apparatus provides for continuous replenishment of substrate at an operator defined rate. Both of these functions are essential for adequate assessment of the function of a kidney (or liver or pancreas). Failure to replace lost volume will lead to an increase in hematocrit and protein concentration in the remaining plasma or serum. Returning the excreted fluid to the perfusate, as is commonly done, complicates or does not allow organ function to be measured. This also permits waste products such as urea, shed cells, lactic acid, bile salts, and digestive enzymes to accumulate in the perfusate, which is not desirable.

In laboratories that use small animal organs for physiological evaluation, additional problems arise. For instance, an organ that is floating randomly in a solution prior to being attached to the perfusion apparatus presents a number of problems. The vessel orientation, prevention of vessel twisting, matching of vessel length to the length of the perfusion apparatus venous and arterial connection lines, and organ handling with minimal touching and surface friction all are difficult with a floating organ.

Normothermic perfusion apparatus previously described does not control environmental temperature. All the emphasis had been on controlling temperature of the

perfusate. Previously described apparatus has not considered the significance of ambient humidity. Further, there has been no consideration of organ surface drying. Finally, previous apparatus has not provided a sheltered environment to minimize the chance of bacterial contamination.

There is considerable literature on the perfusion cooling of heart-beating cadavers for multiorgan procurement. It has been found that such perfusion can adequately preserve internal organs without conflict. Application of this technique after cardiac arrest, rather than before cardiac arrest, could help to salvage organs that are now abandoned. It is urgent, therefore, that similar techniques be available to be used after cessation of cardiac function. Thus, organs that are currently lost can be salvaged. Standard heart-lung machines are capable of initiating in vivo perfusion and reoxygenation after cardiac arrest, however they cannot follow through on reversing damage. Further, heart-lung machines cannot document the in vivo perfusion conditions or connect this information with the conditions used for, and the results of, subsequent regeneration of each procured individual organ in vitro.

SUMMARY OF THE INVENTION

The instant invention provides a perfusion apparatus and method for perfusing organs which overcome the above noted deficiencies of conventional perfusion apparatuses and methods. Specifically, the invention provides a perfusion apparatus and method with an automatically controlled and monitored fluid system. The system includes a controller and monitors for the organ and the fluid. In preferred embodiments, the controller measures the viability of an organ, as well as measuring and controlling various characteristics of the perfusion fluid. The perfusion system may be made of blood-compatible materials, although fluids other than blood can be used. Useful perfusates include fluids selected from the group comprising blood, modified blood, ordinary

crystalloid, modified crystalloid and oxygen-carrying crystalloid. In addition, pharmaceutical agents, physiological agents and fixatives may be used.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a schematic drawing of the perfusion system according to an embodiment of the invention.

Figure 2 is a drawing of a vascular immobilization/organ transport platform according to an embodiment of the invention.

Figure 3 is a drawing of an embodiment of the invention permitting the apparatus to be used in vivo prior to, during, and after transfer of an organ perfused in vivo to the in vitro apparatus.

Figures 4A, 4B, and 4C are drawings of a three-way occluding valve according to an embodiment of the invention.

Figure 5 shows an alternative embodiment of a three-way occluding valve.

Figure 6 displays the results of a recent experiment as an example of the functioning of the device and of the software that operates it.

Figure 7 displays the results of another experiment as a further example of the functioning of the device and the software that operates it.

Fig. 8 illustrates graphs representing parameters monitored during blood perfusion of an isolated kidney for the experiment of Fig. 7.

Figs. 9-16 illustrate graphs representing various parameters monitored during blood perfusion versus time.

Fig. 17 illustrates an alternative embodiment of a vascular immobilization/organ transplant platform.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

Overall Features

In embodiments, the perfusion apparatus has a modular construction that is easy to assemble and disassemble. The perfusion apparatus also provides for a disposable, sterile and optionally low-volume (to reduce the volume of blood or fluorocarbon needed) fluid system

that is suitable for use on human or animal organs that may be later transplanted. Fluid systems having different volumes may be provided for different sized organs. The modular fluid system permits a sterile and economical construction from known components.

Preferred embodiments include an organ transfer platform, preferably a position-adjustable platform. The platform eliminates the need for direct handling of the organ. The platform prevents the organ from changing orientation after removal from the donor and before placement in the perfusion apparatus. The platform presents the organ in a flexible manner which facilitates connection of the organ to the apparatus. The platform can also prohibit unwanted and potentially hazardous vascular motions. Artery and vein positions can be adjusted according to the natural requirements of each organ. These positions are then firmly held in position avoiding any danger to the organ during attachment to the perfusion system.

Fluid can be moved through the system by a pump which is non-traumatic to blood or other synthetic or natural perfusion fluids such as emulsions. A computer controlled adjustable piston-cylinder blood pump is preferred. However, other types of pumps can be used if desired. The pump controls the pressure of the infused fluid in the fluid system.

Furthermore, the apparatus can control independently the infusion of nutrients, drugs and alternate perfusates into the fluid in the fluid system, thus permitting the organ to receive vital and beneficial material. For example, the controller can actuate valves and/or pumps connected to liquid sources. This is especially beneficial if the organ is damaged so that the addition of the nutrients or pharmaceutical agents can resuscitate the organ.

The use of a blood pump also allows for a pulsating perfusion to simulate the natural in vivo environment of the organ. The pressure pulse wave can be

readily adjusted manually and/or by computer as desired, from a low pulse amplitude to a high pulse amplitude. The use of a computer to control the perfusion apparatus and process permits the mean blood pressure, systolic blood pressure, and diastolic blood pressure, along with other parameters of interest to be determined, controlled, displayed, and logged in real time, in any manner desired. This is especially beneficial in determining the status and viability of the organ.

The invention further permits facile, sterile connection of a disposable circuit to the blood pump and the sensors of the fluid system. A facile calibration of fluid sensors and pressure transducers is readily achieved with the computer controlled perfusion apparatus.

The apparatus can also monitor organ functions. Organ function such as urine flow rate (when pertinent), vascular resistance, oxygen consumption, acid and CO_2 production and the like can be continuously or intermittently measured. This permits a ready viability assessment of the organ based on the functioning of the organ. Further, simultaneous measurement and display of pO_2, pCO_2, pH, temperature, organ weight (edema) and the like can be accomplished by the computer controlled perfusion apparatus. Electrical cross-talk can be avoided, which is advantageous.

The invention can further provide automated, pre-arranged urine and blood collection. Fraction collectors permit renal clearance calculations to be carried out either acutely, if in-house serum/urine analysis capability exists, or during subsequent organ storage. Similar calculations can be made for other organs. In addition to being more convenient and less disruptive to the perfusion than manual collection methods, automated sample collection by the computer controlled perfusion apparatus avoids technician errors.

The computer controlled perfusion apparatus can also continuously replace "lost" fluid volume. Fluid lost as urine (or bile, pancreatic duct fluid, etc. depending

on the organ) can be replenished at the same rate volume is lost. Uncompensated loss of volume will result in altered hematocrit, plasma protein concentration, perfluorochemical emulsion concentration, etc. The continuous replacement can be accomplished by sensing the lost volume directly or indirectly, and activating an appropriate pump or pumps to supply additional solution to the perfusion system. This permits avoidance of the recycling of excreted fluids which may damage the organ.

The device is also capable of continuously replacing substrates in the perfusate or fluid at the rate they are consumed by the perfused organ. The replacement can be based on standard rates of substrate utilization or on rates of substrate utilization previously measured in similar organs. Substrate replacement and volume replacement can be accomplished independently of each other.

The computer controlled system can be adjusted to permit either a gradual or abrupt change in the perfusate. One example of a gradual change would be gradual replacement of a low-viscosity crystalloid perfusate with leukocyte and platelet depleted blood. An abrupt change could include a switch from blood to saline and/or a switch from saline to fixative for experimental runs, with collection of fixative or other perfusate in an auxiliary reservoir.

The perfusion apparatus can challenge organs by infusing pharmaceutical, physiological or rescue agents under the control of the controller. The controller can control the onset time, administration rate, rest periods, etc. of this infusion.

Further, the perfusion apparatus can be used to perfuse a cadaver donor. The perfusion apparatus can then accommodate one or more organs from the donor as organ procurement proceeds and is completed.

The perfusion apparatus or portions thereof, especially including the connection to an organ, may be enclosed in a sterile or semi-sterile climate-controlled

enclosure, cabinet or incubator. The enclosure can preferably maintain an internal air temperature with a heating element and/or internal humidity using small amounts of sterile water. Further, an additional organ box, into which an organ transport platform fits, can shelter the organ from contact with condensed water and from bacterial contamination. The enclosure may be sterilized between uses, such as with disinfectants and/or an internal UV light. The enclosure is preferably configured to position the organ to be visible at all times during the perfusion. The enclosure may have doors to render the organ accessible from one, two or more sides. Transparent surfaces of the enclosure may be covered to protect personnel during UV sterilization.

Furthermore, the apparatus may be provided with microscopy/image analysis capability. This permits a non-invasive viability assessment to be based on morphological and cell physiological measurements, for example fluorescent or colored tag measurements.

The advantages of a perfusion apparatus for organ resuscitation are numerous. The perfusion apparatus can include a pump that will not be affected by hypotensive agents. Any desired intervention, no matter how unphysiologic, can be accomplished using such a apparatus. For example, the operator can use hypertonic perfusates to reverse cell swelling, high potassium perfusates to restore cell potassium, or perfusates containing red blood cells or perfluorochemicals, but devoid of platelets and leukocytes, to avoid thrombi and inflammatory reactions while supplying oxygen and time to permit organ lesions to heal. Further, it is possible to be able to switch from one type of perfusate to another for, for example gradually raising hematocrit as organ resistance falls, under computer control.

Components

The components used to construct the perfusion system may be selected from the list of parts and materials provided in Tables 1 and 2. A separate

interface board (the PBM-4 Minarik Process Control Module, Shingle Gibbs, Inc. Baltimore, Md) ran the Harvard Apparatus Blood Pump and was directly mounted inside the blood pump housing. A custom electrical system was supplied by Eldex Inc. to allow drop detection by the computer.

The Perfusion System

Fig. 1 presents a schematic diagram of the perfusion system. The fluid-conducting elements of the system may be prefabricated as one disposable pack that can be introduced into the equipment as a unit. The system may also be composed in whole or in part of modules that can be connected by using sterile Luer Lok™ fittings and piercing fittings, such as blood bag and blood bag tube spikes. For example, blood bag tubing spikes may be used to insert lines into mating inlet and outlet ports of pump 30. Optionally, a male or female Luer fitting may be used to effect this connection.

When the perfusate is blood or modified blood, the tubing used is preferably blood-compatible tubing such as blood bag tubing, Tygon® tubing, or Silastic® tubing.

The organ 1 is atraumatically supported on a special organ holder and transport platform 2. Platform 2 organizes the renal vessels after organ excision and prior to perfusion in vitro. The organ transport platform 2 is placed into an organ container 3. Container 3 provides general protection for the organ and may, when the organ is electrically excitable, such as a heart, be used to facilitate mounting of electrodes used to monitor and/or pace electrical activity. It also prevents platform 2 from contacting the top loading balance 4. This further ensures the sterility of organ 1.

The organ container 3 and the organ platform 2 are designed to allow free access to the organ 1. Openings in the container wall allow cannulae to enter and exit. Platform 2 and container 3 may be combined into a single unit if desired. The outer container 3 could be eliminated or replaced by a piece of stretched parafilm.®

A top-loading balance 4 measures and continuously monitors the weight of organ 1 to estimate the amount of tissue edema. For small organs, such as rabbit kidneys used in biological experiments, the organ 1 is weighed prior to placement on the transport platform 2. When the organ 1 and accompanying equipment are positioned on the top-loading balance 4, the weight is tared to zero. Total organ weight is then monitored as the initial organ weight plus the change in weight (organ edema) registered by the balance 4 after taring. For large human organs, it may be sufficient to tare the weight of the container 3 and platform 2 to zero and make a direct measure of organ weight, because minor factors such as influences of the cannula position on organ weight may be negligible for large organs.

Organ weight is registered by a direct connection between the balance 4 and a serial port of a computer 5. The computer 5 controls and documents the perfusion. The data could be transmitted by other equivalent means, if desired. The incoming data are input as character strings. The character strings are analyzed with a stored string recognition algorithm to isolate the information desired. This permits the desired information to be converted back to a number (the number of grams of organ edema) and passed to the portion of the program that requests the information. The weight data, and all other data, can be deposited into a spreadsheet or into a spreadsheet format by the computer 5.

A jack 45 is provided to lift or lower the balance 4 and the associated organ 1, platform 2, and container 3 to adjust the elevation because organs vary in size, shape, and vascular orientation. The jack 45 may be motorized and may be controlled by a joystick located external to cabinet 42. Cabinet 42 encloses the organ and permits the temperature-controlled, humidity-controlled environment to be established.

The inputs and outputs to the organ include an arterial inflow line 6, venous outflow line 7, and

secretion/excretion line 8. Fluid or perfusate is delivered to the organ 1 from the arterial line 6 and drained from the organ 1 by the venous line 7. The vein is cannulated in order to permit measurements of oxygen consumption, carbon dioxide production, and blood acidification as indices of the fundamental metabolic balance sheet of the organ 1. Correction for losses of oxygen and carbon dioxide in any secretions or excretions is not significant because the volume of secretions from organs, including urine flow in kidneys, is normally such a small fraction of total organ blood flow and because the oxygen and CO_2 carrying capacity of these secretions is low in comparison to blood. Oxygen consumption, CO_2 production, and acid production are calculated from measurements of gas tensions and pH in the arterial line 6 at sensor bay 15 and in the venous line 7 at sensor bay 16 using standard formulas.

Sensor bays 15 and 16 are CDI sensor heads (made by 3M Health Care, Tustin, California) or an equivalent. CDI heads also measure arterial and venous temperatures, to check on temperature control and uniformity within the perfusion cabinet 42. The CDI heads are connected to a readout device 17. Readout device 17 sends a signal to the computer 5 via a serial port. CDI sensors or equivalents are selected because they are able to collect the data without electrical interference.

In Figure 1 reservoirs or containers 11 and 12 may be provided to hold fluid. The fluid is significantly different in composition from the blood or other solution travelling through the system. A waste collection reservoir 14 may be provided to drain undesired perfusate.

Manual or computer-actuated valves 9, 10, 13, 18, 33, 34, 35, and 45 are provided in the fluid system. Elements 18 and 45 are pinch-type solenoid valves (such as the Model 100P2WNC valves sold by Bio-Chem Valve Co., East Hanover, NJ) capable of blocking lines 48 and 49.

Valve elements 9, 10, 13, and 33-35 are preferably three-way (rather than two-way) valves. These valves may

either occlude the tubing by pressing on the tubing from outside, in the manner of valve 18, or by conventional flow diversion mechanisms where perfusate actually travels through channels in the valve. We term the later, a flow-through valves (e.g., valves such as NR Model 648T033 teflon-lined solenoid valves, Neptune Research, Maplewood, NJ). These channels may be selected either manually, as with a three-way stopcock, or by computer via a selection of the position of an internal piston in the valve.

Flow through valves have two disadvantages: a) the material in the valves must be blood compatible, and b) the tubing leading to and from the valve cannot be continuous when using valves that are currently commercially available, because there is no way to insert a continuous tube into such a valve. Conventional valves made with blood-compatible material may be used, despite these disadvantages.

In a preferred embodiment, specially-designed valves shown in Fig. 4A-4C or 5 are used that allow continuous tubing to be employed. Therefore, a single disposable tubing pack can be used. This allows flow diversion by external compression so the valve itself need not be made of a blood-compatible material.

Line 49 permits the sampling of blood. Line 49 is preferably of narrow diameter to avoid the loss of perfusion pressure upon the activation of valve 18. Also, valve 18 is to be activated intermittently, rather than continuously for each blood sample collection for the same reason.

Typically, reservoirs 11 and 12, valves 9, 10, and 13, and dumping site 14 are used where the perfusion is to result not in transplantation, but in chemical fixation. The present invention may be used not only to evaluate and resuscitate organs for clinical use, but also to conduct experiments on organ resuscitation and evaluation after other procedures.

To use reseervoirs 11 and 12 the blood/perfusate pump 30 is turned off at a defined moment. Valves 9 and

13 are activated immediately thereafter so as to permit fluid from reservoir 11 to drain into arterial line 6, proceed through the organ 1, and exhaust to drain site 14 through valve 13. Typically, reservoir 11 will contain saline to flush blood from the organ 1 that would otherwise become trapped in the organ by the fixative in 12. After the saline rinse from reservoir 11, valve 10 is activated to allow the contents of reservoir 12 (typically fixative) to flow through the organ instead of the contents of reservoir 11.

Fluid from reservoirs 11 and 12 may drain into the organ 1 under the influence of gravity. Alternatively, the fluid may be propelled by a pump (not shown) placed between 9 and 10.

Reservoirs 11 and 12 are preferably within the temperature-controlled cabinet. Therefore, fixation can proceed without changing organ temperature.

The positioning of valves 9 and 13 between the organ and sensor bays 15 and 16 results in the organ being fixed without contamination of the sensor heads, perfusion pump, pressure transducer, or other vulnerable and reusable system components with fixative.

Upstream of arterial sensor bay 15 is an entry site for arterial infusion line 48. Line 48 permits the delivery of rescue drugs from syringe 46 under the control of computer-actuated syringe pump 47. The use of a syringe and a syringe pump is not critical, another type of equivalent reservoir and fluid metering system being acceptable. The infusion entry site is guarded by pinch valve 45 preventing the contamination of line 48 by perfusate and preventing unwanted contamination of the active perfusion circuit with drug.

Upstream of line 48 is the exit point for arterial blood sampling. This point is governed by pinch valve 18 and leads to the fraction collector 20 and the associated drop counter 19. Drop counter 19 is used to ensure collection of a consistent volume with each blood collection (typically about 2 ml). As noted above, line

49 is chosen to have a narrow diameter to limit pressure drops upon actuating valve 18. Valve 18 is also, as noted above, actuated and deactivated several times for each blood collection, if need be, to further ensure adequate maintenance of arterial perfusion of the organ during blood sample collection.

Line 49 is upstream of line 48 to avoid contamination of blood samples with infused drugs, and loss of infused drug into collected blood samples.

Upstream of line 49 is a junction 21, which leads both to the pressure transducer 22 and toward the source of perfusate. Transducer 22 sends a signal to, and receives excitation from, an interface 9b, between it and the computer 5. The computer calibration for the pressure transducer may be checked at any time by reference to mechanical pressure transducer 24. Transducer 24 is protected by a diaphragm 25 to avoid contamination with perfusate.

Element 26 is a coarse blood filter. Element 27 is a coil of Silastic^{R} tubing that serves as a gas exchange surface (oxygenator and CO_2 remover). Tubing 27 is surrounded by a gas pocket 28 in which oxygen from tank 29 flows to introduce oxygen and carry off carbon dioxide. It is not necessary and can even be detrimental to supply CO_2 gas to tubing 27. 100% oxygen or a mixture of oxygen and nitrogen is therefore recommended.

A three-way valve 33 is upstream from the oxygenator. The pump 30 will draw solution either from bag 31 or bag 32, depending on whether or not valve 33 is actuated. By switching from bag 31 to bag 32 during a perfusion and actuating valve 13, it is possible to abruptly and essentially completely replace a given perfusate with another. On the other hand, by activating valve 33 intermittently, the perfusate in bag 31 can be gradually blended with the perfusate in bag 32. This can lead to either a full or a partial substitution of the first perfusate with the second perfusate over a predetermined time. If desired, additional bags and

valves could be added to enable additional complexity using the same approach.

Valve 35 is complementary to valve 33. When the perfusate of bag 31 has switched to the perfusate of bag 32, any volume replacement or nutrient replacement should also be transferred from bag 31 to bag 32 using valve 35. Of course, the fluid delivered for volume replacement from the reservoir 37 by the pump 36, and the vehicle for the nutrients in syringe 38, as delivered by computer-actuated syringe pump 39, must also be compatible with the perfusate composition of the bag 32. If it is not compatible, a similar three-way valve or a flow-through 3-way valve, would be need to be interposed between 36 and 37 to permit a different volume replacement solution to be chosen instead of the solution in reservoir 37 to ensure compatibility with the new perfusate. Fortunately, the volume of infused nutrient solutions is expected to be small enough, and the vehicle solution is expected to be universal enough, to preclude the need for separate nutrient solution infusions for separate perfusates most of the time.

Valve 34 is similar to valve 35 in that it controls which bag, 31 or 32, receives incoming fluid. In valve 34, the incoming fluid is venous return from the perfused organ. Note that bags 31 and 32 are located vertically below or at the level of the organ 1, not substantially above it. This is to avoid creation of a substantial venous outflow pressure that would slow perfusion and promote organ edema.

It would be desirable to measure venous pressure to ensure that it remains within the desired limits. This measuring capability can be incorporated in a manner similar to the inclusion of the arterial pressure transducers 22 and 24.

The secretion collection line 8 leads to drop counter 40 in route to fraction collector 41. The drop counter 40 is a standard unit used with laboratory fraction collectors. Its output is directed to the

computer 5 though a custom electrical circuit specially designed to permit its voltage response to be input to the computer at high speed so that voltage peaks and valleys could be resolved and interpreted as overlapping pulses. Each pulse represents one drop. This procedure is necessary to allow drops to be resolved and accurately counted, particularly when the drops are closely spaced. This is important when perfusing kidneys, for example, since urine flow tends to be pulsatile, rapid volleys of drops interspersed with slower drop rates being normal. The results were converted from drops per unit time into volume per unit time using a measured calibration factor and optionally into volume per unit time per gram of original organ weight.

The overall perfusion system is divided into upper cabinet 42, which is temperature and humidity controlled, and lower cabinet 44, which is less controlled. Humidity is controlled in cabinet 42 with a humidifier 50. The temperature is maintained through standard thermosetting mechanisms known in the art. If necessary the reservoir 37 and even pump 36, may be included in cabinet 42 to ensure temperature stability. However, the volumes delivered from pumps 39 and 47 are not sufficient to create thermal stability problems.

The Organ Handling System

The invention provides for an organ transport/handling platform 2, as shown in Fig. 1, to prevent twisting of the vessels during transport to the apparatus. The platform also prevents motions of the vessels during attachment to the apparatus. The vessel-organizing features of the platform are of particular value in handling small organs in biological experiments. The organ-cradling feature is of equal value for both large human organs and small animal organs.

The organ-handling platform is described in Fig. 2. The platform 216 includes an organ cradling area 217 and a cannula control area 218.

The cannula control area 218 consists of a main body 201 and a retainer bar 204. The retainer bar 204 mates with the main body 201 by means of the hinge 205 and the thumbscrew 206. Bar 204 may be pivoted up to allow organ cannulae to be laid on the floor 219 of the main body 201 without restriction of any kind on the lateral position or rotational orientation of these cannulae. The bar 204 may then be lowered and locked in place with thumbscrew 206 or other suitable means to immobilize the cannulae and/or their associated tubing in the previously-selected, preferred positions. The immobilization is attained by placing vertical downward pressure on the cannulae and/or tubing according to the relative heights of the cannulae or tubing cross-section and the gap 207, which is determined by the construction of the recess 219 receiving the bar 204. The recess 219 is in the lateral wall of base portion 201. The height of the gap 207 is selected to meet the requirements of the specific cannulae and/or tubing used. It should be chosen to apply sufficient pressure to immobilize the tubing and/or cannulae without collapsing the tubing/cannulae significantly. The vertical elevation of the recess floor 222 and the bar 204 are selected to suit the type and size of the organ being perfused.

Often the vertical height of the vessels emerging from an organ 213 is not what is expected. In this case, since the floor 219 of base 201 is horizontal and at a fixed elevation, an angle can be imposed between the horizontal tip of the cannula near the organ and the organ vessel leading from the organ to the cannula tip. This angle may alter the flow through the organ and damage the organ vessel. To avoid this problem, the base 201 is constructed to pivot within the organ cradle part 217. Thus a straight line path from the cannula tip to the organ can be achieved by altering the horizontal disposition of the cannula. This is attained by incorporating pin 203 and lever 202 in the cannula control base 201 and by incorporating thumbscrew 210 and holes 209

in the organ cradling part 217. The pin 203 is positioned in the hole 209 immediately beneath the retaining thumbscrew 210. The lever 202 protrudes through the remaining hole 209. When screw 210 is disengaged, the lever 202 may freely swivel element 218 to adjust the cannula angle as desired. When the desired orientation is attained, the thumbscrew 210 is tightened to prevent further motion. Other means of securing the position of part 218 within part 217 are also acceptable.

The organ cradle part 217 contains a fine mesh or other deformable surface 211. The surface 211 allows the organ to sag slightly into recess 212. This cradles the organ in a hammock-type fashion and thereby reduces the force on any given surface element of the organ required to support the organ's weight.

Aperture 220 in wall 208 of organ cradler 217 allows the secretion cannula 221 to emerge from the device. Cannulae 221 is permitted to emerge at the position that best suits the anatomical requirements of the particular organ being perfused. The position of the ureter, for example, can be of extraordinary importance in governing urine flow rate.

Fig. 3 describes an application of the apparatus for use in vivo. Lines 6 and 7 are made long enough to be extended sufficiently from cabinet 42 to be placed into the aorta and vena cava, or into one or more individual organ vessels (e.g., renal artery and vein) of the donor. Separate (single or multiple) individual fluid flow systems originating with arterial lines 45 are provided for collected organs.

A single pump may be used for a cadaver and for all perfused organs. However, if flow is to be registered for each individual organ, a separate pump will be required for each organ.

The first collected organ can be transferred to the cabinet while continuing cadaver perfusion. Activation of that particular system can be accomplished using the types of valves described above. Second, third,

or later organs can be added to the machine as they are procured. The lines 6 and 7 of Fig. 2 can be excluded from the system when all organs have been collected. In Fig. 3, the reservoirs, pump, oxygenator, and tubing are enlarged to be appropriate for use on a cadaver.

Valve Construction

The construction of the three-way externally occluding valves 9, 10, 13, and 33-35 is described with respect to Figs. 4 and 5.

The tubing to be occluded is configured in a "T" pattern. In Fig. 4, tubing segment 402 leads to the organ while tubing segment 403 leads from the main perfusate source, and tubing segment 401 leads from the bags 11 and 12 as seen in Fig. 1. Thus, the primary or normal flow is from 403 to 402, with 401 being blocked. The secondary flow direction is from 401 to 402, with 403 being blocked.

In Fig. 4A, four arc-shaped fingers 404-407 are similarly shaped and shown end-on. The fingers 404-407 project past the segments of the tubing. Each finger 404-407 is attached to a rod 408-411. Each rod 408-411 can be rotated about its axis by a suitable means (not shown). In the normal flow pattern, rod 408 is rotated clockwise and rod 409 is rotated counter-clockwise. This movement forces fingers 404 and 405 toward each other, thereby occluding tubing segment 401. Tubing segments 402 and 403 remain unaffected. To switch to the secondary flow pattern, the rod 409 is rotated clockwise and rod 411 is rotated counter-clockwise. This brings fingers 405 and 407 together, thereby occluding tubing segment 403. Rod 404 is also rotated counter-clockwise back to its "rest" position, shown in Fig. 4A. This removes deformation of tubing segment 401 without affecting tubing segment 402.

Finger 406 is not used during a perfusion, but may by used during preparation of the circuit prior to perfusion. With fingers 405 and 407 in their rest positions, rotating finger 404 counter-clockwise and finger 406 clockwise occludes the tubing segment 402, while segments 401 and 403 remain open. This

configuration may be used to flush segment 401 with perfusate to remove air prior to attaching bags 11 and 12. In addition, simultaneous occlusion of segments 402 and 403 may allow bags 11 and 12 to be attached to the system without unwanted fluid flow from segment 401 to either 402 or 403.

Alternatively, finger 406 and rod 410 could be replaced by a support rod or platform for supporting the segment 402, in other embodiments. Similarly, fingers 406 and 407 could be joined together to form a single finger operated by a single rod, in another embodiment.

The valve of Fig. 4A is preferably operated by the computer 5. Each finger 404-407 is operated independently. Fingers 404 and 405 can assume one of three positions. Fingers 406 and 407 can assume one of two positions. A four way valve could also be employed where fingers 406 and 407 would have a third position to occlude tubing in the direction of area 413.

Fingers 404-407 are attached to rods 408-411 in part by an end-to-end abutting arrangement and in part by an adapter segment 412. This arrangement is visible in Fig. 4B.

Fig. 4C shows a different version of the valve. The fingers 404-407 are shaped like the four smoothed quadrants of a circle. The fingers 404-407 continue to be mounted on the ends of the rods 408-411.

Fig. 5 shows another embodiment of the three-way occluding valve. Here, the tubing segments 501, 502, and 503 are occluded by direct pinching between pincher elements. For example, pinch elements 515 and 516 can occlude tubing segment 502.

Alternatively, three independent pinch valves similar to valve 18 of Fig. 1 could be used. These valves are preferably modified to minimize dead spaces between the tubing junction between segments 502, 503 and 504 and the sites of the occlusion.

RESULTS

Fig. 6 presents the perfusion data for a recent experiment using a rabbit kidney. The data are shown in the format displayed on our monitor in real time. All graphs have the same time base and are displayed as a series of parallel strips. The organ used in the tests was a rabbit kidney. The kidney was perfused with leukocyte and platelet depleted rabbit blood with some hemodilution with a saline/nutrient solution containing hydroxyethyl starch to combat edema.

Trace 1 shows organ weight demonstrating minimal edema formation with perfusion time.

Trace 2 shows that pressure was excellently controlled by the computer. The computer registered systolic (upper thin line), mean (middle heavy line) and diastolic (lower thin line) pressure continuously. The pressure was initially elevated to the target 90 mmHg level gradually to avoid pressure overshoot. Pressure was maintained constant in Trace 2 despite continually improving blood flow, as evidenced by Trace 3. The continually improving blood flow is evidence of organ damage reversal and increased organ viability.

Urine flow rate is shown in Trace 4. The urine flow rate shows continuous improvement after about 40 minutes.

Trace 5 shows the temperature being maintained at the 35-36 degree Fahrenheit target range. This range was chosen because slight hypothermia is known to favor repair in vivo and is advantageous. Any temperature from about 20 degrees to about 37 degrees Fahrenheit may permit organ function to be determined and improved.

The kidney rapidly consumed oxygen which was dissolved in the solution, as shown in Trace 6. This is also evidenced in the calculated oxygen consumption for the kidney as shown in Trace 9. Trace 9 shows more oxygen consumption between time zero and 60 minutes, than after that time. This suggests that some oxygen consumption is being used for organ repair during the first 60 minutes.

Although the partial pressure of carbon dioxide was high and remained so during the experiment (evidently due to an error in the gas selection for this experiment), the production of carbon dioxide by the kidney is clearly visible in Trace 7 in that the venous carbon dioxide tension was higher than the arterial carbon dioxide tension.

Trace 8 shows that the pH was maintained near 7.4. This was maintained despite the high carbon dioxide, as seen in Trace 7.

Trace 10 shows a steady rise in the hematocrit toward the normal level. This was despite volume additions as indicated in Trace 11.

The volume addition was conducted manually in this experiment. This was done to test the ability of a human operator to judge the correction of the system volume. The volume additions shown represent isolated additions rather than a continuous addition, which is possible with a computer. Although the human operator succeeded in approximately stabilizing hematocrit after about 30 minutes, it is apparent that computer control could have been more effective throughout the experiment than was the human operator. Furthermore, the significant increase in hematocrit observed was despite volume replacement, indicating that failure to replace lost volume would have led to much more significant rises in hematocrit, perhaps bringing hematocrit to the point where blood viscosity would have presented serious problems for the perfusability of the organ.

Further experiments of the blood perfusion apparatus were conducted with rabbit kidneys, and a description is now provided. The blood perfusion apparatus allows a kidney be perfused with blood in a closed system for 2 hours with automated control of various parameters. In the experiments, eight freshly harvested kidneys were perfused and used as a control.

Blood perfusion apparatus

A physiological environment is recreated in the blood apparatus. Blood is pumped by suitable pump, for example by a Harvard pulsatile blood pump, through a closed system of blood compatible tubing. The blood is stored in a 250 ml blood collection bag, for example, by Fenwal on a venous line. The blood circulates at constant pressure through the kidney, whose artery, vein and ureter have been canulated. Pressure is detected through a pressure transducer, for example, PX23 by Ohmeda, which signals a computer that in turns sets a pumping rate for the blood pump.

The blood is oxygenated by passing through a coil of siliconic tubing in an oxygen saturated chamber. The gas composition of the blood is continually recorded by a blood gas analyzer, such as a CDI blood gas analyzer. The system is equipped with a 40 micron microaggregate filter, for example, by Baxter on the arterial line.

The volume of urine produced is measured by a drop counter data, for example, by Eldex, and an equivalent volume of fluid is returned to the blood bag. Thus, the perfusate composition is kept constant. A modified Weinberg solution reservoir is connected to a 0.22 micron filter, for exmaple, by Millipore, through a return line to the blood bag. A roller pump, for example, by Masterflex, is programmed so the pump rate is monitored according to the volume of urine produced. When added to the blood, the Weinberg solution allows to keep the electrolytes and hemotocrit at a constant level.

The system described above, is positioned on top a water bath, and is covered. This maintains the required normothermic temperature and humidity.

Perfusion Fluids

Blood: At least 100ml of blood was drawn from the ear median capillary of three different rabbits in a mixture of asprinc/heparinc (10mg and 1000 mg units). Platelets and white cells were removed by centrifugation to minimize their interference with the perfusion. The hemotocrit is reduced to 25%-30% by adding modified Weinberg solution.

Weinberg solution: The Weinberg solution has the following composition

	g/l	M
KCl	0.225 g/l	3 mM
KH2PO4	0.136 g/l	1 mM
NaCl	5.5 g/l	94 mM
NaH_2PO_4	0.69 g/l	5.75 mM
$NaHCO_3$	1.26 g/l	15 mM
Na Acetate, $3H_2O$	1.7 g/l	12.5 mM
Na Lactate	1.41 ml/l (1.68 g/l)	15 mM
Butyric Acid	0.55 g/l	6.3 mM
$MgSO_4, 7H_2O$	0.246 g/l	1 mM
Ca Gluconate	0.43 g/l	1 mM
Alanine	0.089 g/l	1 mM
Glycine	0.375 g/l	5 mM
Creatinine	0.040 g/l	0.35 mM
Glucose	1.0 g/l	6 mM
HES	30 g/l	3% (when required)

Osmolarity 300 mOs/kg (305 mOsm/kg when HES)
pH = adjusted to 7.4

Kidneys

The right kidney was harvested from a rabbit donor by standard procedure (see B.S. Khirababi and G.M. Fahy, Cryobiology 31, 10-25, 1994). The kidney is flushed with an Eurocollins (EC) solution to remove residual blood, and stored in EC until it is connected to the blood perfusion apparatus, but not more than 2 hours. The renal artery, vein and ureter are canulated, and the organ is placed on a stage platform. Before connection, the kidney is flushed with modified Weinberg solution to remove any residual EC. When the kidney is connected to the perfusion system, the pressure is activated and is gradually increased to 90 mmHg.

Perfusion conditions are maintained as sterile as possible, for example, by autoclaving material, or cleaning up the disconnect parts with Tergazyme, rinsing them with MilliQ water and keeping them in closed containers.

After blood perfusion has been conducted for 2 hours, the kidney is disconnected and immediately flushed with EC. The kidney is stored in EC until retransplantation, but no longer than 40 min. The

transplantation is done on the left hand side of the rabbit after nephectomy on the left side kidney.

Perfusion

The kidney is perfused with 25-30% hematocrit blood oxygenated at a pressure of at least 120 mmHg. The perfusion pressure is maintained and controlled at 90 mmHg for 2 hours. Urine is collected by a fraction collector, and the volume measured. Fluid is returned to the blood in an amount equal to the measured volume. Blood samples are collected during the perfusion at time interval of 0, 15, 45, 75, 105 and 120 min. to determine the blood chemistry.

A computer file is generated after the perfusion and data can be graphed as in Fig. 7.

Parameters evaluated

This system allows parameters to be followed, such as blood flow, arterial pressure, urine flow, oxygen consumption. It also allows observation of cortical changes. Oxygen consumption was also evaluated. At precise time intervals, blood and urine samples are collected and their chemistry is an indicator of renal function, such as glomerular filtration, protein exclusion and tubular reabsorption of various chemical substances.

After transplant the rabbits are carefully monitored and their blood creatinine, urea, and other electrolytes. Histology was also performed at the time the rabbit is sacrificed. Glomerula and proximal and distal tubules appearance is observed. The results are summarized in Tables 3 and Figs. 8-16.

A futher embodiment of an organ platform A, as shown in Fig. 17, is provided for the kidney. The organ lies on a soft mesh 2000 that covers an aperture 2001 in a platform 2002. The mesh 2000 is bonded to the platform 2002. A renal artery cannula tubing 2100 and cannula (not shown) generally snaps into a recess 2003 in a positioning bar 2004. This assures a favorable orientation of the artery with respect to the kidney. A further recess 2005 includes two cylindrical pathways through positioning bar

2004. A venous cannula (not shown) or venous cannula tubing 2200 can be snapped into one of two closely-spaced positions of recess 2005 to assure a favorable orientation of the vein with respect to the kidney. The circular cross-section of recess 2003 and the cylindrical pathways of recess 2005 permit the cannulas to be rotated clockwise or counterclockwise as needed to prevent twisting of the artery or vein, respectively.

Further, the positioning bar 2004 may contain an extra channel 2006 for ducts such as, in this case, the ureter cannula. Legs 2007 position the platform up off an underlying surface to reduce the chance of contamination from any nonsterile part of the underlying surface in sliding relationship to tilting bar 2008, which would be the platform, along side rail 2009. Tilting bar 2008 would alter the angle of the kidney slightly to better orient the renal artery and vein with respect to apertures 2003 and 2005. Tilting bar 2008 would be closely approximated to platform 2002 and need not be in the form of a bar. It could also be wedge-shaped or possess other cross-sectional shapes to conform to the shape of different types of organ.

Table 1 = Small Parts List for Composing the Invention

Items	Chemical Nature	Initial item when modification have been added	Characteristics	Model #	Vendor
TUBING CONNECTION					
Luer Male	Polycarbonate		connectors 3/16'	RMB03041	Gish Biomedical
Luer Female	Polycarbonate		connect to tubing 3/16'	RMA07195	Gish Biomedical
Taper Luer Coupler	Polycarbonate	fit MLLR-9	for male/male connection	MTLC-9	Value Plastics
Taper Luer Square Ring	Polycarbonate	fit MTLC-9	for male/male connection	MLLR-9	Value Plastics
Male Taper to Barb for 1/8'	Polycarbonate	fit RMLLR-9	connector for male rolling luer lock	MTLRZ30-9	Value Plastics
Male Luer Round ring	Polycarbonate	fit FTLT-9	for manifold / tubing connection	RMLLR-9	Value Plastics
Female Taper T - Luer	Polycarbonate	fit RMLLR-9		FTLT-9	Value Plastics
Silicon tubing: to join some connectors	Silicon Medical Grade		1/8' ID - 1/4' OD 3/16'	T 5715-121	Scientific Products
Blood Bag Tubing	PVC	Taken from Transfer set	Tubing used for blood circulation	4C2243	Fenwal, Baxter
Teflon Connector for the filter	Teflon	Taken from pack	Fit 4C7730	RCM 71M	Stericon
Y- Luer adaptor	PolyUrethane		for pressure & fluid connection	MCY 300	Medcomp
Cannula	Teflon	Silicon tip	1.7 mm ID	500	Neostar
Teflon sleeve	Teflon	from Stericon set	to connect filter	RCM 71 M	Stericon
Transfer set	PVC	with 2 spikes	to connect pump filter/oxygenator	4C2243	Baxter
Filter	PVC	40 um transfusion	after oxygenator	4C7730	Baxter
Filter	PVC	.22 um	for fluid sterility	Millex-GS	Millipore
Filter	PVC		Leucofilter	RC-50	Pall
Bag 150 ml	PVC		for blood Collection	4R2001	Baxter
Bag 300 ml	PL1240		As Nutrient Bag		Baxter
Tube 12 ml	Polypropylene	100 x 13 mm case of 500	Urine Collection	55.516	Sarstedt

Table 2 = Major Components

Part	Material	Description			
FOR: BLOOD PUMP					
Blood Pump				1403	Havard
Sleeve	Polycarbonate				Shop Made
Fittings for blood bag spikes	Polycarbonate				Shop Made
Balls for Ball Valves	Teflon Coated Stainless Steel		5/16" balls .001" Teflon coating	# BNMX-5	Small Parts
FOR: CABINET					
	Plexiglass	4 side panels 1 top cover 2 doors+ 2 hinges			Shop made
Shelf	Plexiglass	to support the scale			Shop made
Frame	Aluminum				Shop made
FOR: PRESSURE CONTROL					
Pressure transducer		Gould Statham		P23 XL	Omeda
Diaphragm Dome		Gould Statham		TA 1010D	Omeda
Manometer		Tycos	Pressure display	7050-15	Baxter
Transducer protector	Acrylic	Connect Manometer		N2413/ FOP273	Kendall Mc Gaw
FOR: OXYGENATOR					
Case	Plexiglass	one cap fitting on one cylindrical sleeve			Shop Made
Threaded female Luers	Polycarbonate	Provide connection to tubing		FTLLB230	Value Plastics
Silicone Tubing	Silicon	To provide gas exchange		T 5715 -26	Scientific Products
Y connectors	Polycarbonate	to hold the silicon tubing	ID=3/32"	Y-220-9	Value Plastic
BLOOD GAS ANALYZER					
Blood Gas Monitor	Continuous arterial	blood gas		400	CDI - 3M
Gas Calibrator	Gas I 5.5% O2 Gas II 30% O2	Calibrate sensors			CDI -3 M
Sensor Heads					CDI-3 M
Cells	Membrane				CDI - 3M
Silastic® Tubing	Silicon	To connect Membrane to system		Silicon Tubing	Scientific Products
Luer connection	Polycarbonate		Luer RMLLR-9		Value Plastics
FOR: URINE/BLOOD COLLECTION					

Part	Material	Description			
Fraction collector, drop counter, and excitation/readout circuit for data transfer to computer	Polycarbonate		100 x 13	UFC	Eldex
Collection tube				55 516	Starsted
COMPUTER					
Monitor				VGA	
Computer				XT	IBM
Interface				MINI 16	Industrial Computer Source, Inc.
				AOB6-P	Industrial Computer Source, Inc.
Software				Misc. Housings	Proprietary
THERMOCOUPLE	Copper/constant	For cage Temperature			Shop made
BALANCE				Galaxy 4000	Ohaus
Nutrient/drug infusion pumps (syringe pumps)				355	Sage Instrument
Volume replacement pump (roller pump)	Masterflex			7016.20	Cole Parmer

Table 3

Rabbit	Perfusion Duration	Time Peak creatinine	Peak creatinine	Recovery Time	Hemolysis	Hct	RBF	Uflow/ RBC presence	GFR peak	Time peak GFR	GFR at end	VFR peak	VFR at end
	min	days	mg/100ml	days	%	%	ml/min	ml/min	ml/min				
3	105	4	16	0	high	<30%	60	1.5	4.63	45	2.7	68.4	38
6	120	3.75	11.9	10	6%	<30%	40	1.8 RBC	3.78	75	2.2	91.7	52.6
8	130	1.875	9.4	11	2%	<32%	50	1.8 No RBC	3.95	45	2.08	81.8	52.6
9	120	2	8.5	8	1%	<32%	50	1.6 Few RBC	3.79	120	3.33	75.8	53.2
Mean		1.9375	8.95	9.5									
2	85	1.2	6	4	slight Cortical Hemorrage	<30%	30	1.5 RBC	5.61	60	4.5		
7	120	1	4.8	7	1.40%	>30%	50	3.0 few RBC	4.36	45	2.9	70.2	50
5	120	1.7	3.9	6	little	<30%	30	1.4 few RBC	2.23	105	1.58	78.9	51.3
4	105	2.5	3.4	6	none	<32%	50	1.5 No RBC	3.64	75	2.45	67.2	62.5
Mean		1.6	4.525	5.75									

Summary of some blood perfusion parameters (Pressure, Renal blood flow (RBF), Hematocrit (Hct), Urine flow (Uflow), linked with some renal function Glomerular filtration (GFR), Volume reabsorption (VRF)) and general observations such as hemolysis at the end of the perfusion.

Table 4

Summary of the renal function of the perfused kidneys and lists the GFR, Protein exclusion, Na, K, Cl and glucose reabsorption

	Min.	GFR Glomerular Filtration Rate ml/min	FF Filtration Fraction (%)	VFR Volume fraction reabsorb (%)	Tubular Reabsorption [Na] (%)	[K] (%)	[Cl] (%)	[Prot] (%)	[Glu] (%)
Rabbit 2									
	15	0.810667	4.548397	60.52532	61.34301	23.54571	54.69108	98.90351	56.15976
	45	1.587636	5.077639	70.27027	69.0485	17.0045	61.24517	99.19649	91.32883
	75	4.528333	11.49747	67.27273	68.77005	9.002217	60.19656	99.20177	82.33035
	85	4.55	12.13333	71.42847	74.74654	-27.9503	67.66917	99.20635	50.63492
Rabbit 7									
	15	3.110909	5.795062	62.06897	65.35632	24.86737	61.72414	100	90.27409
	45	4.364286	6.6357	70.21277	72.69504	50.66489	67.7305	100	95.31915
	75	3.287143	5.052016	46.15385	51.53846	37.99534	45.17483	100	79.38903
	105	3.091667	4.233951	48.57143	53.87013	33.87755	48.09524	100	66.76923
	120	2.871111	4.192358	52.63158	56.99064	35.08772	52.18471	100	71.57895
	130	3.12	4.541333	50	52.76074	35.71429	48.13084	100	69.56522
Rabbit 5	15	1.2896	4.232909	75.96154	76.732	17.14403	73.90697	99.27154	88.25393
	45	1.805	5.652775	78.94737	82.70198	0.478469	80.39927	99.36204	97.66082
	75	2.01	5.28441	66.66667	77.22567	11.11111	72.93447	100	99.96377
	105	2.258824	5.269395	57.5	62.68293	28.70968	56.03448	100	99.96205
	120	1.580526	3.834754	51.28205	53.90757	18.80342	45.82228	100	99.60392
Rabbit 4									
	15	2.132	6.055433	75	73.51974	32.44681	69.02655	99.21875	98.68421
	45	2.7755	6.5026	67.21311	70.38604	41.62335	65.47222	100	99.6992
	75	3.644176	6.098829	58.53659	62.88768	34.34959	56.71801	100	99.43587
	105	2.453333	3.615148	62.5	35.34483	35.34483	59.77273	99.16667	95.65972
Mean									
	15	1.835794	5.15795	68.3892	69.23777	24.50098	64.83718	99.34846	83.343
	45	2.633106	5.967179	71.66088	73.70789	27.4428	68.71179	99.63963	96.002
	75	3.367413	6.983181	59.65746	65.10547	23.11456	58.75597	99.80044	90.27975
	100	3.088456	6.312957	60	64.23115	17.49544	57.89291	99.59325	78.25648
	120	2.225819	4.013556	51.95682	55.4491	26.94557	49.00349	100	85.59143
	130	3.12	4.541333	50	52.76074	35.71429	48.13084	100	69.56522

Table 5

Summary of renal function measured by blood and urine chemistry for the group of most successful perfusion.

	Min.	GFR Glomerular Filtration Rate ml/min	FF Filtration Fraction (%)	VFR Volume fraction reabsorb (%)	Tubular Reabsorption [Na] (%)	[K] (%)	[Cl] (%)	[Prot] (%)	[Glu] (%)
Rabbit 8									
	15	1.775429	4.120053	73.07692	78.31699	12.32742	75.54693	100	87.37839
	45	3.9445	6.367883	71.42857	77.798	49.20635	74.46271	100	99.60591
	75	2.227038	3.124445	57.89474	73.11351	39.18129	67.93819	100	98.69103
	105	2.099289	2.847181	52.63158	63.62782	18.01619	60.17699	100	91.73287
	120	2.216667	3.077622	52.63158	59.91903	7.236842	55.88235	98.89841	85.93017
Rabbit 9									
	15	3.123714	7.999622	75.86207	82.26601	-0.30651	78.71473	100	83.78521
	45	2.912813	6.767333	68	75.68	20.59259	72.14815	100	95.21088
	75	3.234	6.626825	64.28571	68.33333	45.71429	63.5989	100	88.65546
	105	3.285	4.553465	53.33333	66.03376	51.53846	62.22222	100	82.5
	120	3.7896	4.94938	58.33333	61.55914	45	57.10784	100	75.46296

Table 6

Summary of renal function measured by blood and urine chemistry for the group of acceptable perfusion.

	Min.	GFR Glomerular Filtration Rate ml/min	FF Filtration Fraction (%)	VFR Volume fraction reabsorb (%)	Tubular Reabsorption [Na] (%)	[K] (%)	[Cl] (%)	[Prot] (%)	[Glu] (%)
Rabbit 3									
	15	3.223579	6.316356	71.21212	69.30564	0.142045	68.12771	100	98.49475
	45	4.632833	7.071167	68.42105	68.42105	56.72515	66.10333	100	95.20993
	75	3.697231	4.967155	59.375	63.94531	54.73214	62.22588	100	89.04718
	98	2.6915	3.598127	42.85714	45.97403	30	44.32234	100	71.96262
	105	2.708875	3.679981	38.46154	42.14648	23.45216	39.51348	100	66.32801

Table 7

Summary of renal function measured by blood and urine chemistry for the group of acceptable perfusion.

	Min.	GFR Glomerular Filtration Rate ml/min	FF Filtration Fraction (%)	VFR Volume fraction reabsorb (%)	Tubular Reabsorption [Na] (%)	[K] (%)	[Cl] (%)	[Prot] (%)	[Glu] (%)
Rabbit 6	15	2.88	10.14978	91.66667	91.55093	59.79167	89.60317	100	98.75731
	45	2.280571	6.194559	73.07692	83.9568	62.44939	79.80769	100	100
	75	3.779286	8.155905	62.16216	66.67263	70.46803	58.04121	100	100
	105	2.1	5.313851	52.38095	56.19048	47.36842	55.60936	100	100
	120	2.744444	5.740463	52.63158	56.08226	53.61842	48.80383	100	98.8008

WHAT IS CLAIMED IS:

1. A method for perfusing an organ with a fluid, comprising:

connecting an organ to a fluid system, the fluid system including at least a controller and a monitor;

flowing fluid from the system into the organ and perfusing the fluid through the organ;

monitoring organ characteristics and fluid characteristics;

communicating monitored organ characteristics data and fluid characteristics data to said controller; and

controlling with said controller at least one member selected from the group consisting of flow, contents and physical characteristics of the fluid as a function of said monitored data.

2. A method according to claim 1, wherein said controller compares said monitored organ characteristics data and said monitored fluid characteristics data to reference organ characteristics data and reference fluid characteristics data, and controls said system to minimize differences between said monitored data and said reference data.

3. A method according to claim 2, wherein said monitored fluid characteristics data includes fluid pressure data and said reference fluid characteristics data includes reference fluid pressure data reflecting a pressure of the fluid needed to perfuse the fluid through the organ, and the controller controls a pump to regulate the pressure of the fluid in the fluid system and in the organ.

4. A method according to claim 1, wherein said system comprises a piston-cylinder blood pump controlled by said controller.

5. A method according to claim 1, further comprising determining viability of said organ with said controller as a function of said monitored data.

6. A method according to claim 1, further comprising displaying the monitored data.

7. A method according to claim 1, wherein said monitored organ characteristics data includes data reflecting an amount of fluid being used by the organ, and the controller controls the amount of the fluid flowing into the organ to replace the fluid being used by the organ.

8. A method according to claim 1, wherein the fluid characteristics include at least one member selected from the group consisting of partial pressure of oxygen in the fluid, partial pressure of carbon dioxide in the fluid, and pH level of the fluid.

9. A method according to claim 1, wherein the organ is a kidney, said monitored organ characteristics data includes data reflecting an amount of urine lost by the kidney as a function of volume loss of the kidney, and the controller controls the amount of the fluid flowing into the kidney as a function of the amount of urine lost by the kidney.

10. A method according to claim 1, wherein the monitored organ characteristics include at least one member selected from the group consisting of temperature of the organ and weight of the organ, and the controller controls changes in said monitored organ characteristics.

11. A method according to claim 1, wherein the method is conducted in vivo.

12. A method according to claim 1, where the method is conducted in vitro.

13. An apparatus for perfusing an organ with a fluid, comprising:

a connector operably connectable to a fluid source and an organ to flow fluid from a fluid source into an organ to perfuse a fluid through the organ;

at least one organ characteristics monitor;

at least one fluid characteristics monitor; and

a controller in data communication with said monitors and controlling at least one member selected from the group consisting of flow, contents and physical characteristics of the fluid flowed through said connector as a function of data received from said monitors.

14. An apparatus according to claim 13, further comprising a position-adjustable organ holder.

15. An apparatus according to claim 13, further comprising a temperature, humidity and sterility controlled enclosure for an organ connected to the connector.

16. An apparatus according to claim 13, wherein the organ characteristics monitor comprises a weight sensor, and the controller comprises a weight comparator and a fluid flow adjustment actuator to adjust flow of fluid through the connector to an organ responsive to a weight change of an organ.

17. An apparatus according to claim 13, wherein said connector comprises an adjustable piston cylinder blood pump a stroke of the adjustable piston cylinder blood pump controlled by the controller to adjust pressure of a fluid flowing through said connector into an organ.

18. An apparatus according to claim 13, wherein said fluid characteristics monitor comprises at least one member selected from the group consisting of an oxygen partial pressure sensor, a carbon dioxide partial pressure sensor and a pH sensor, and the controller comprises at least one comparator and at least one adjustment actuator to adjust a corresponding at least one member selected from the group consisting of oxygen partial pressure, carbon dioxide partial pressure and pH of fluid flowing through the connector to an organ responsive to data received from said fluid characteristics monitor.

19. An apparatus according to claim 16, wherein the organ characteristics monitor comprises a temperature sensor, and the controller comprises a temperature comparator and a flow adjustment actuator to adjust

temperature of an organ responsive to a temperature change of an organ.

20. An apparatus according to claim 13, wherein said controller comprises a comparator that determines viability of said organ as a function of said monitored characteristics.

21. An apparatus according to claim 13, wherein the fluid flow system is disposable.

22. An apparatus for perfusing an organ with a fluid, comprising:

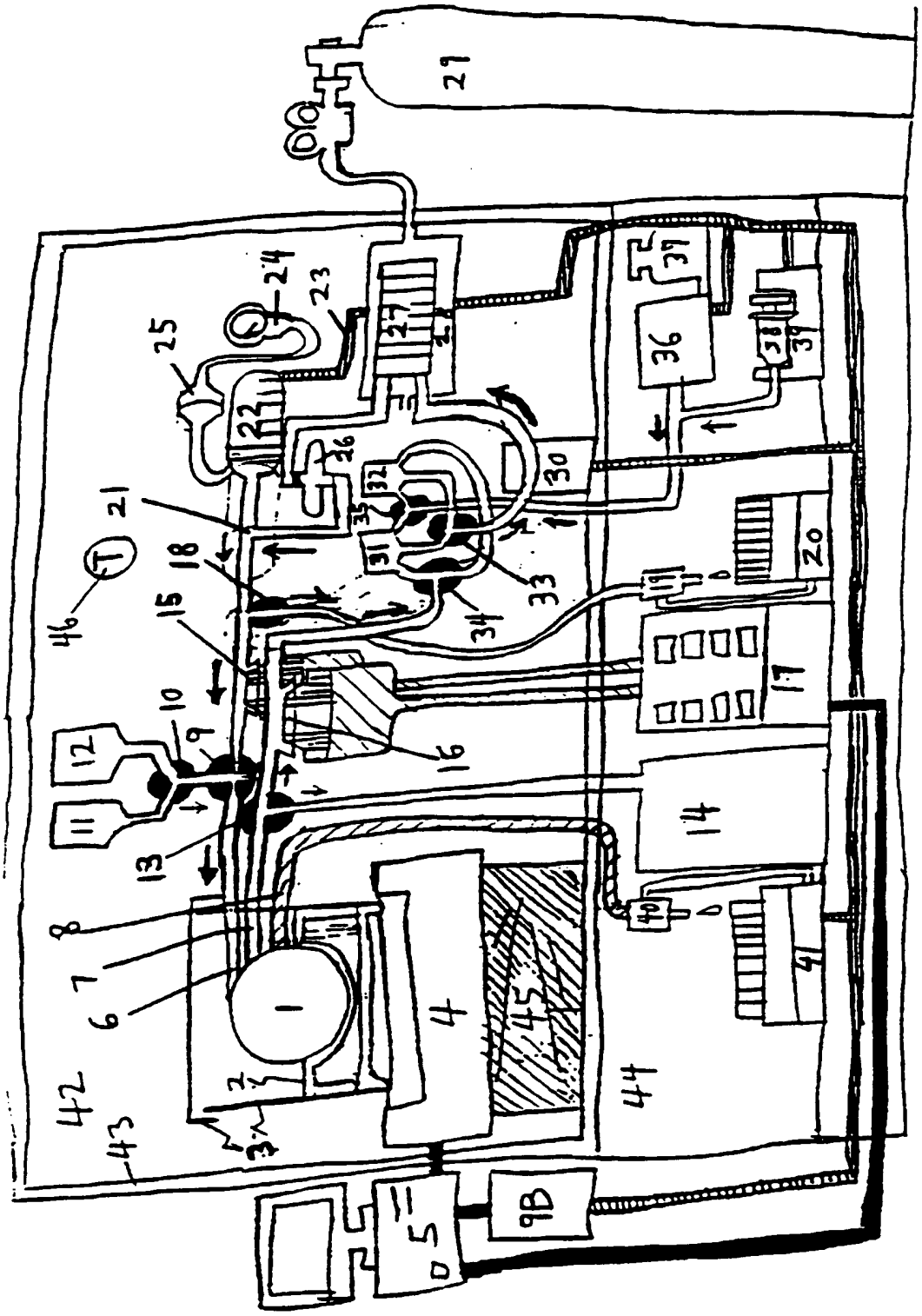
a fluid system including at least a controller and a monitor, and means for connecting an organ to said fluid system;

means for flowing fluid from the system into the organ and perfusing the fluid through the organ;

means for monitoring organ characteristics and fluid characteristics;

means for communicating monitored organ characteristics data and fluid characteristics data to said controller; and

means for controlling with said controller at least one member selected from the group consisting of flow, contents and physical characteristics of the fluid as a function of said monitored data.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12
11
13
16
17
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14
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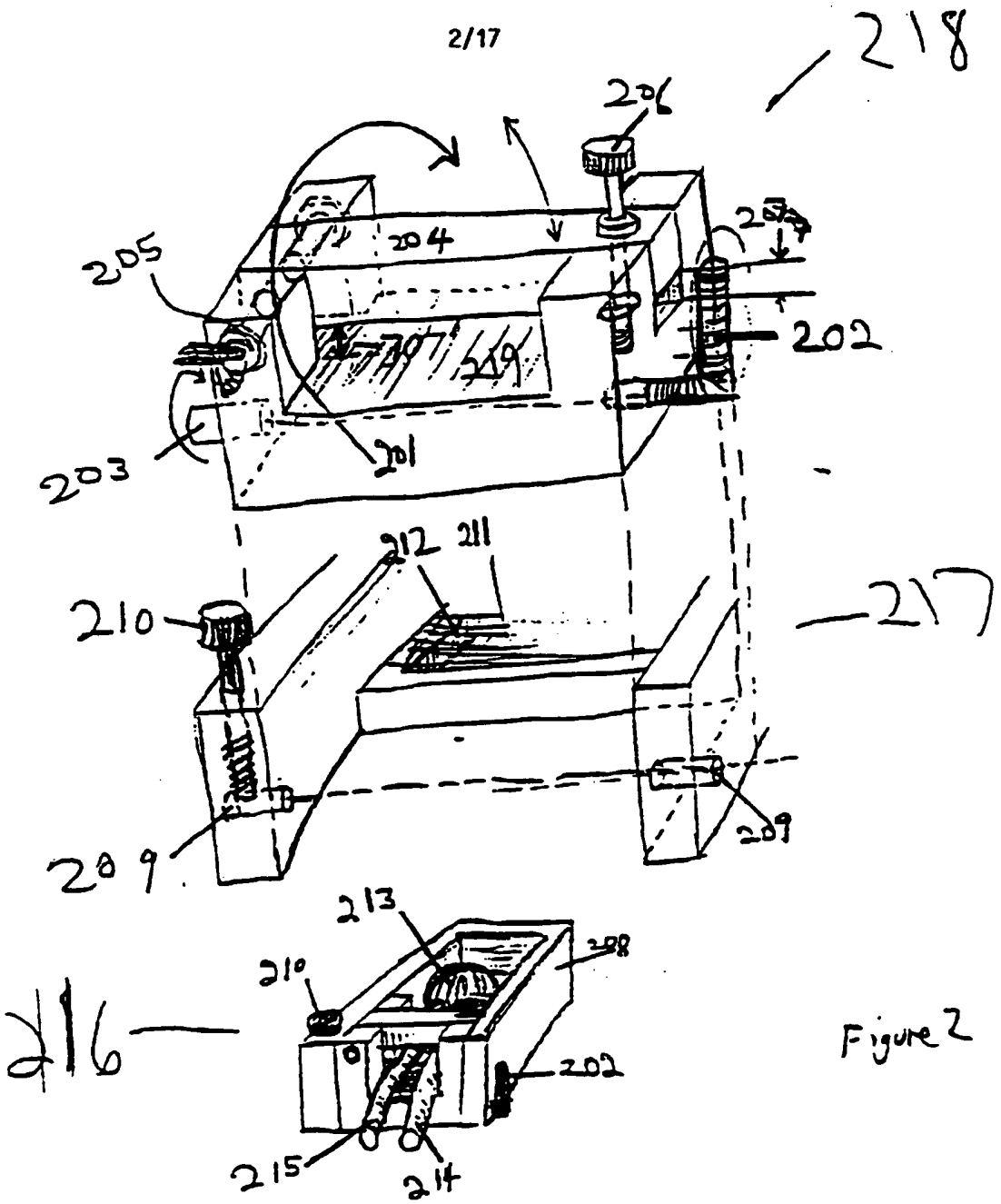
41
8
7
6
1
4
45
42
43
44
3
5
9B

FIG. 1


218
206
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204
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216
202
215
214

Figure 2

Fig. 3

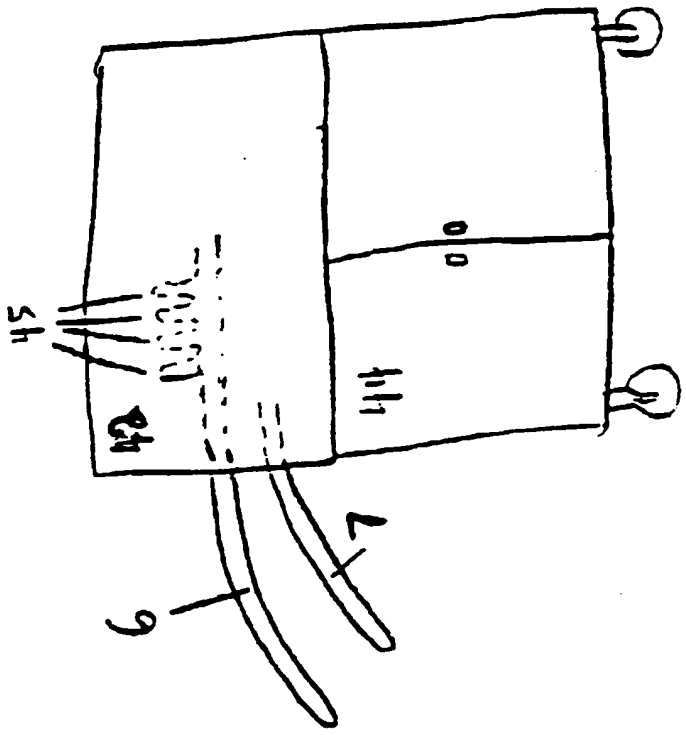


FIG 4A

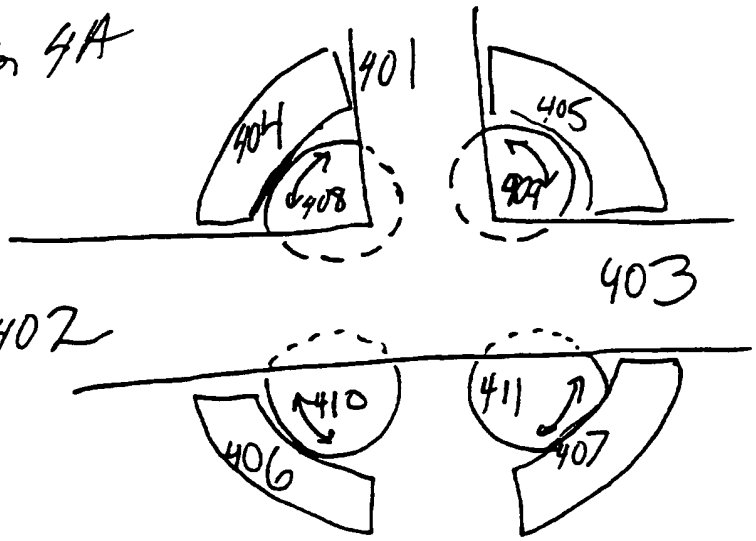


FIG 4B

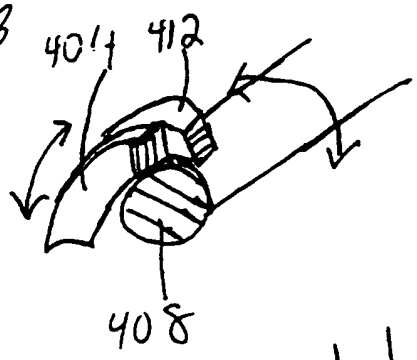


FIG 4C

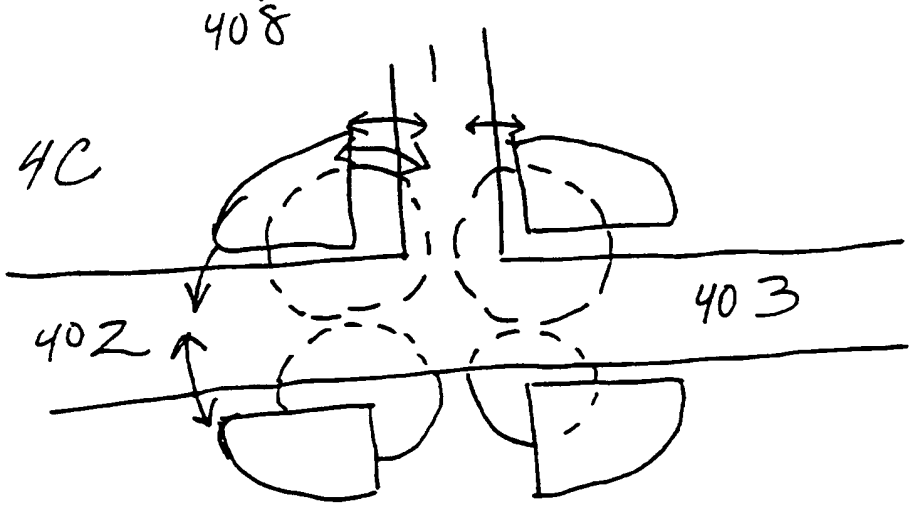
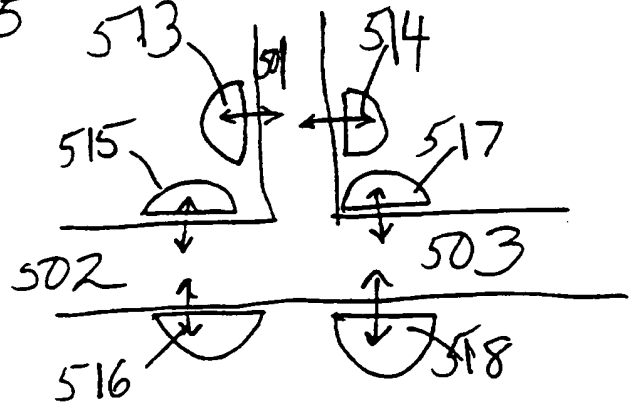


FIG 5



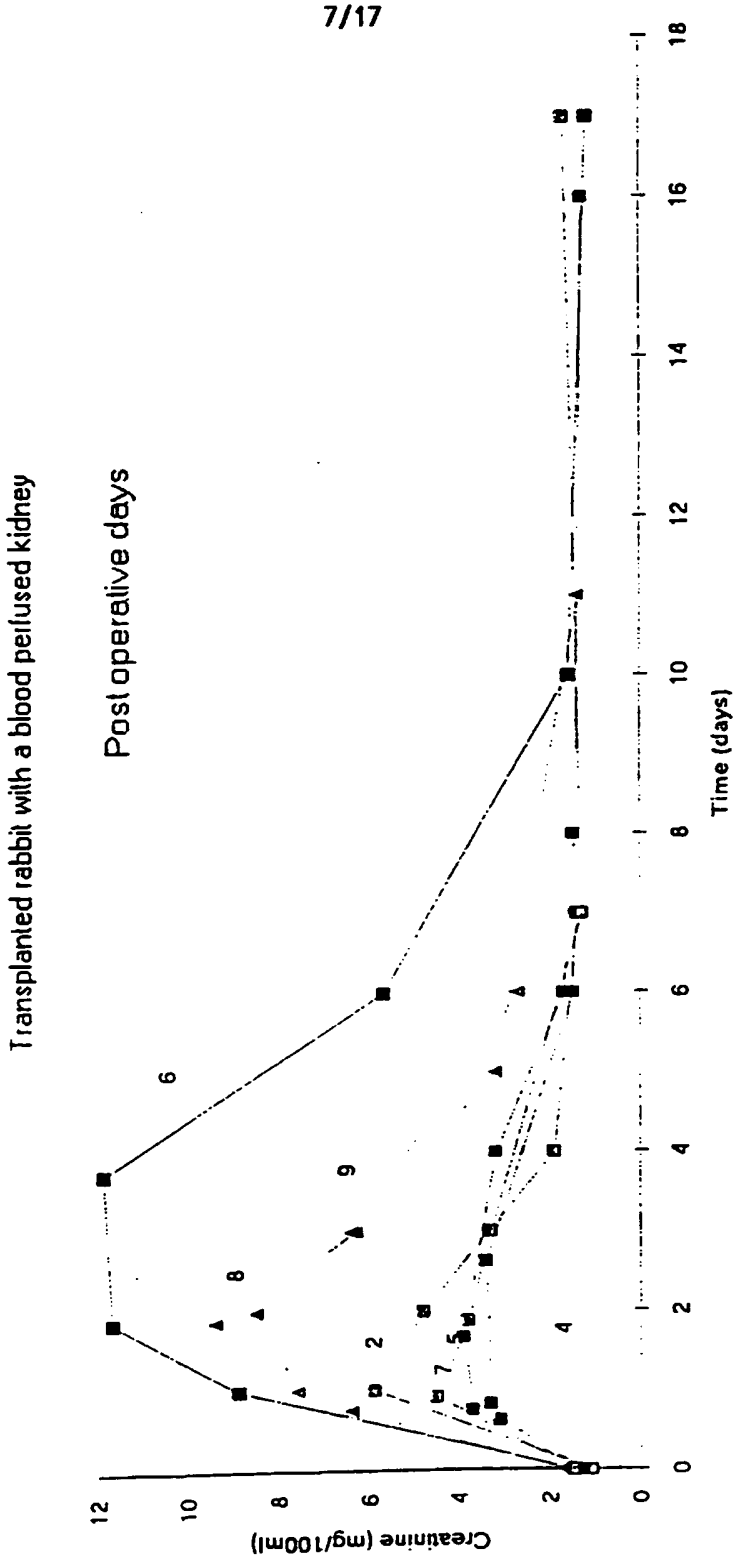

Transplanted rabbit with a blood perfused kidney
Post operative days
Creatinine (mg/100ml)
Time (days)
0
2
4
6
8
10
12
14
16
18

FIG 7

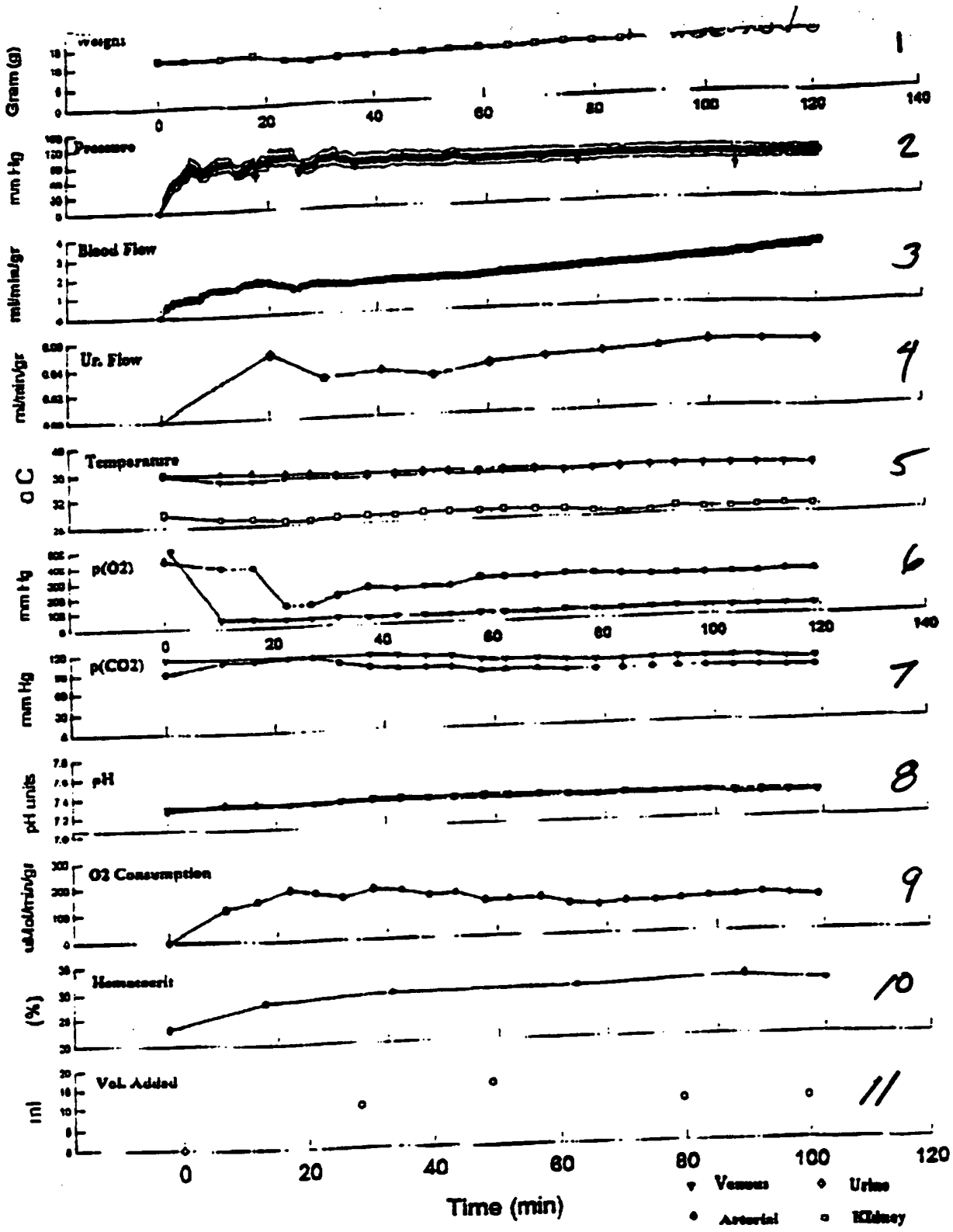

Gram (g)
weight
1
mm Hg
Pressure
2
ml/min/gr
Blood Flow
3
ml/min/gr
Ur. Flow
4
o C
Temperature
5
mm Hg
p(O2)
6
mm Hg
p(CO2)
7
pH units
pH
8
ul/ml/min/gr
O2 Consumption
9
(%)
Hematocrit
10
ml
Vol. Added
11
0
20
40
60
80
100
120
Time (min)
Venous
Urine
Arterial
Kidney

FIG 6

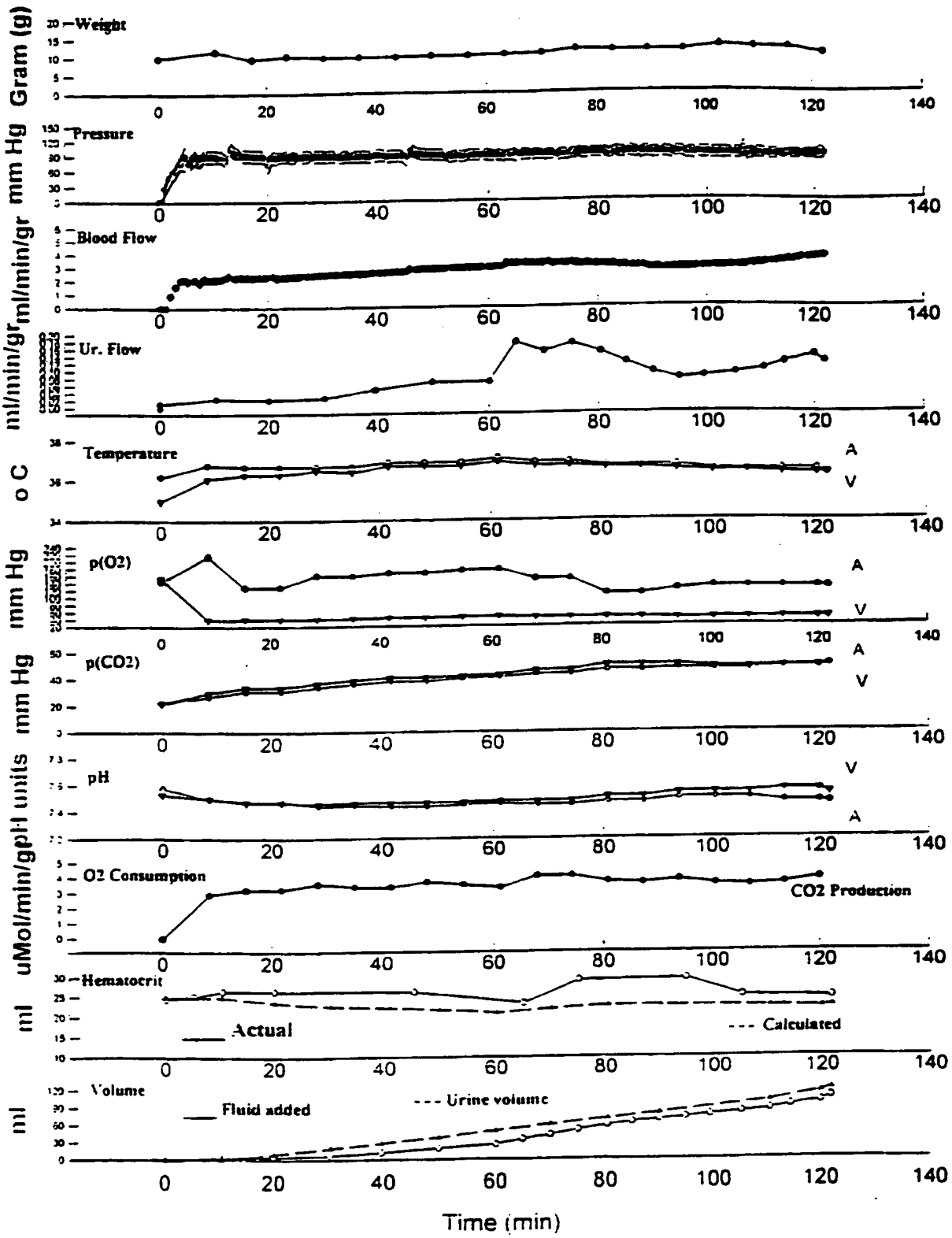

Weight
Gram (g)
Pressure
mm Hg
Blood Flow
ml/min/gr
Ur. Flow
ml/min/gr
Temperature
o C
A
V
p(O2)
mm Hg
p(CO2)
mm Hg
pH
units
O2 Consumption
CO2 Production
uMol/min/gr
Hematocrit
Actual
Calculated
ml
Volume
Fluid added
Urine volume
ml
0 20 40 60 80 100 120 140
Time (min)

FIG 8

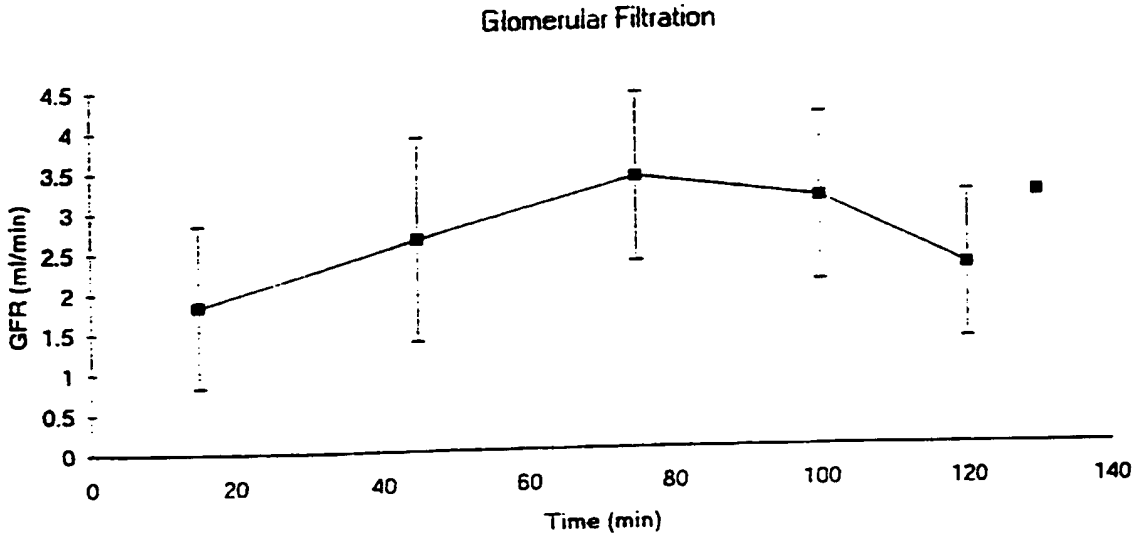

Glomerular Filtration
GFR (ml/min)
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0
20
40
60
80
100
120
140
Time (min)

FIG 9

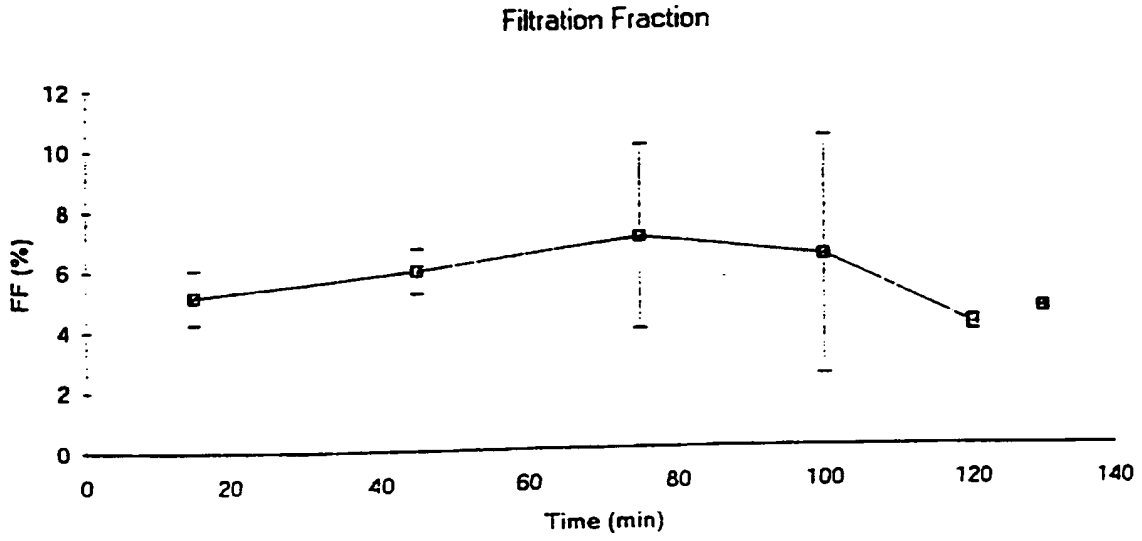

Filtration Fraction
FF (%)
12
10
8
6
4
2
0
0
20
40
60
80
100
120
140
Time (min)

FIG 10

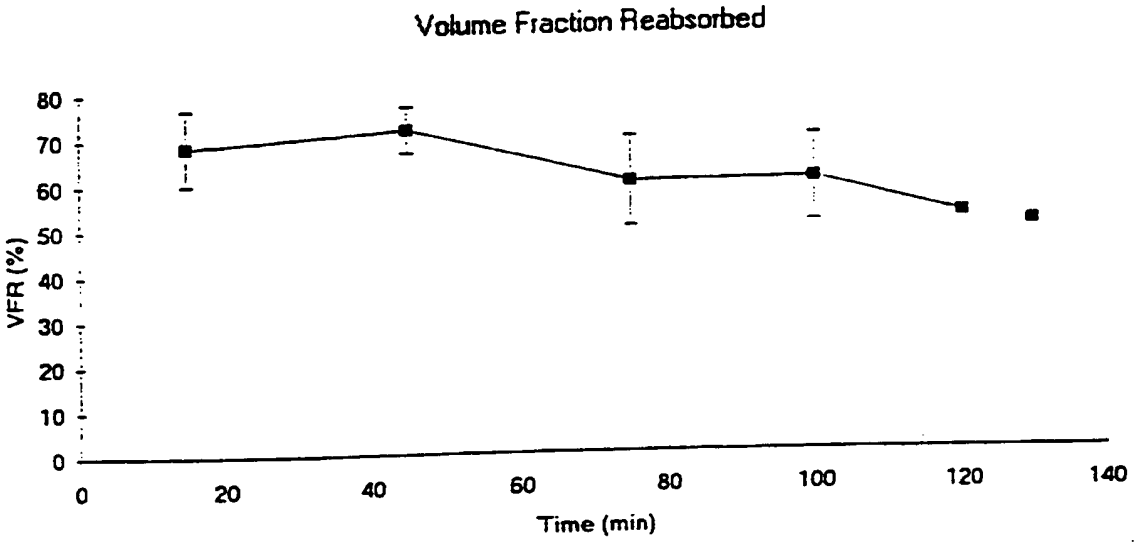

Volume Fraction Reabsorbed
VFR (%)
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
120
140
Time (min)

FIG11

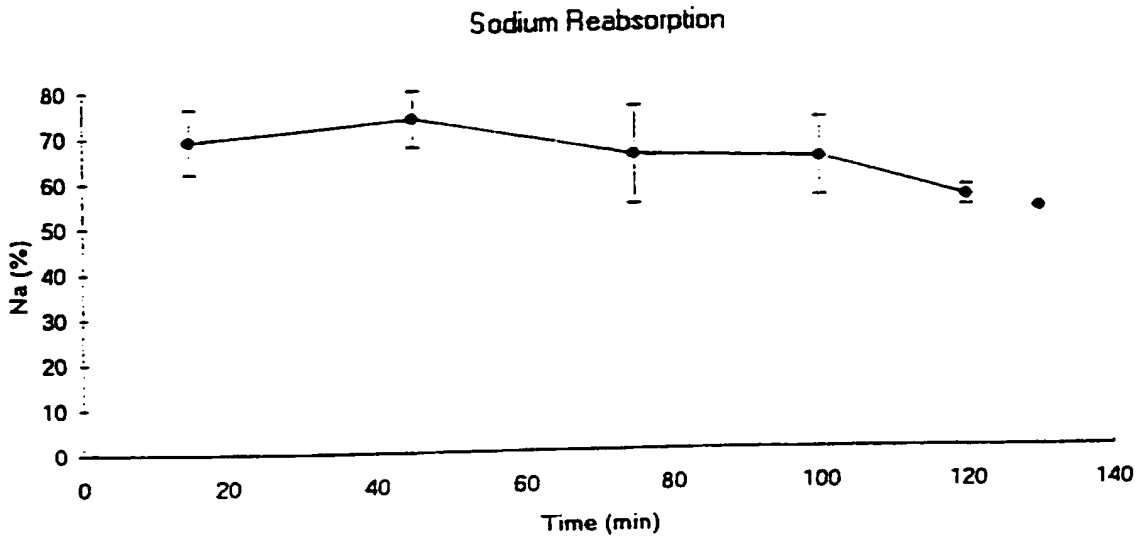

Sodium Reabsorption
Na (%)
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
120
140
Time (min)

FIG 12

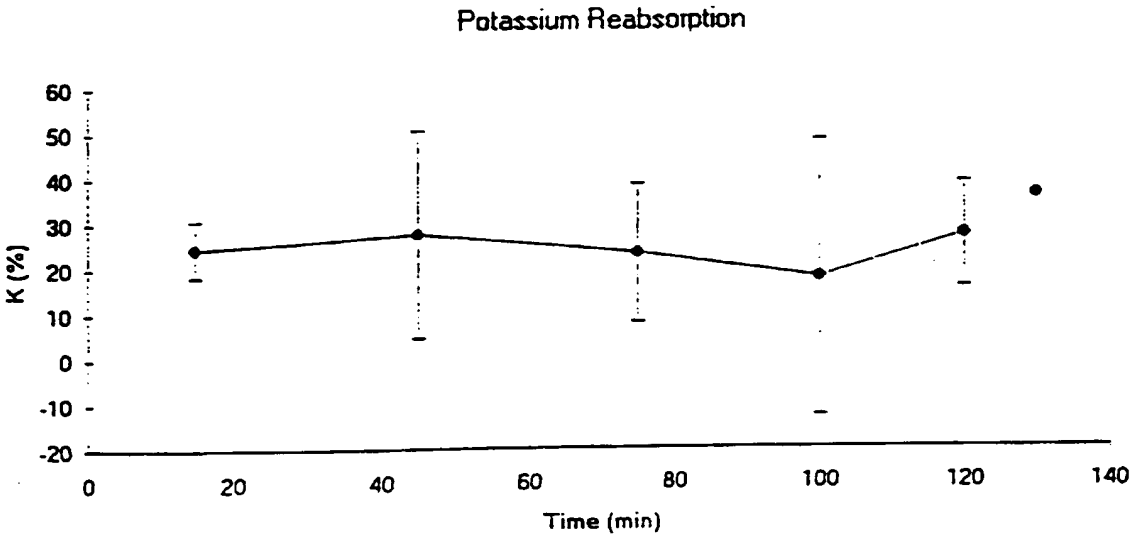

Potassium Reabsorption
K (%)
60
50
40
30
20
10
0
-10
-20
0
20
40
60
80
100
120
140
Time (min)

FIG 13

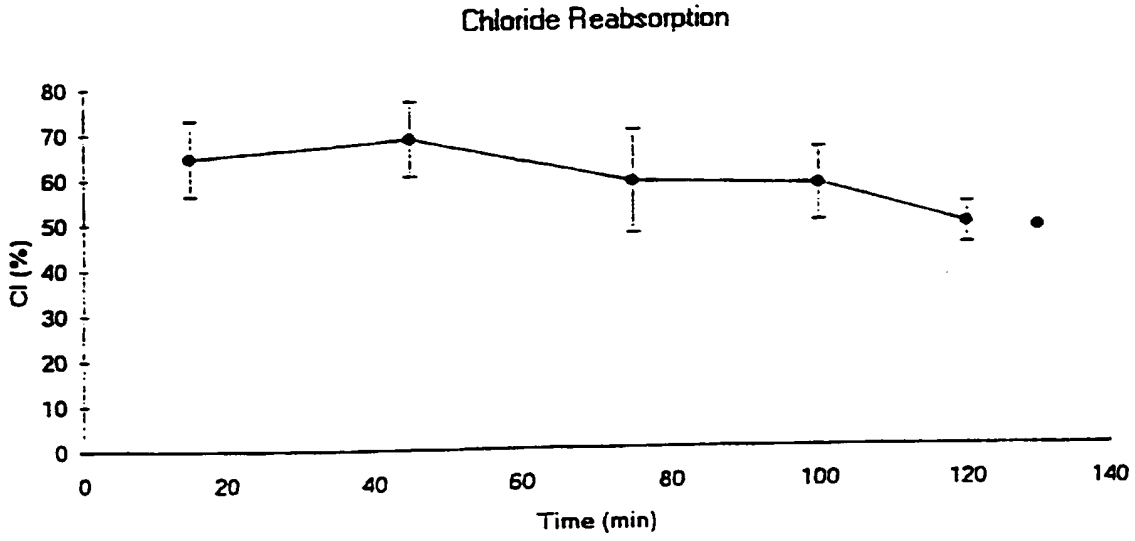

Chloride Reabsorption
Cl (%)
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
120
140
Time (min)

FIG 14

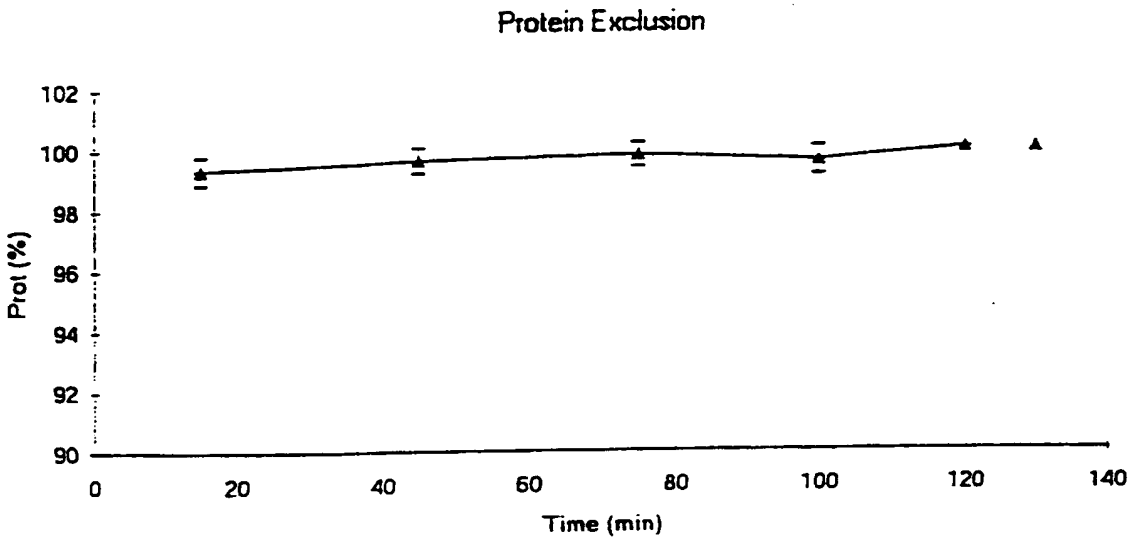

Protein Exclusion
Prot (%)
102
100
98
96
94
92
90
0
20
40
60
80
100
120
140
Time (min)

FIG 15

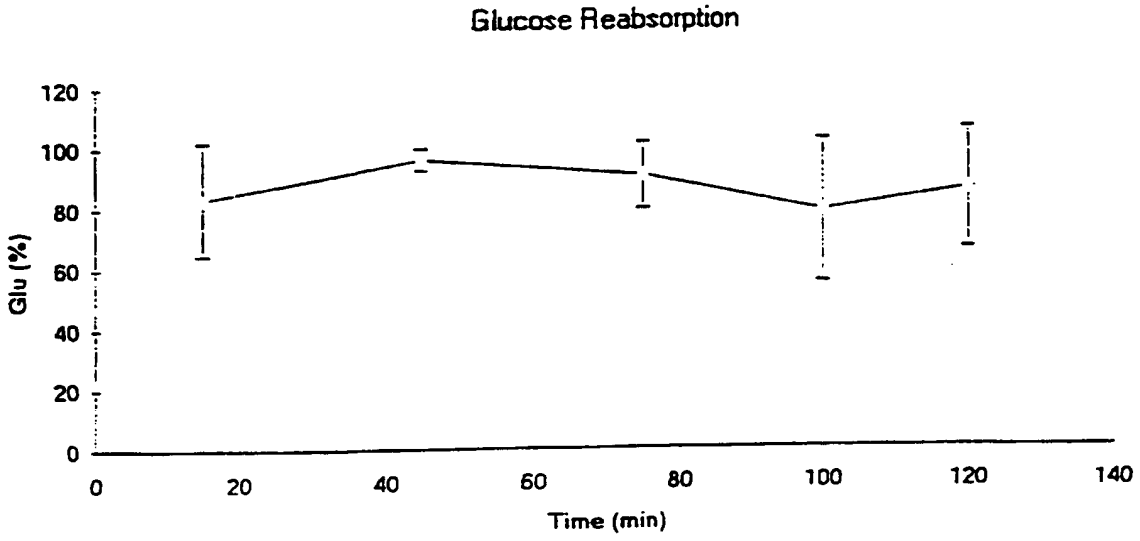

Glucose Reabsorption
120
100
80
60
40
20
0
Glu (%)
0
20
40
60
80
100
120
140
Time (min)

FIG 16

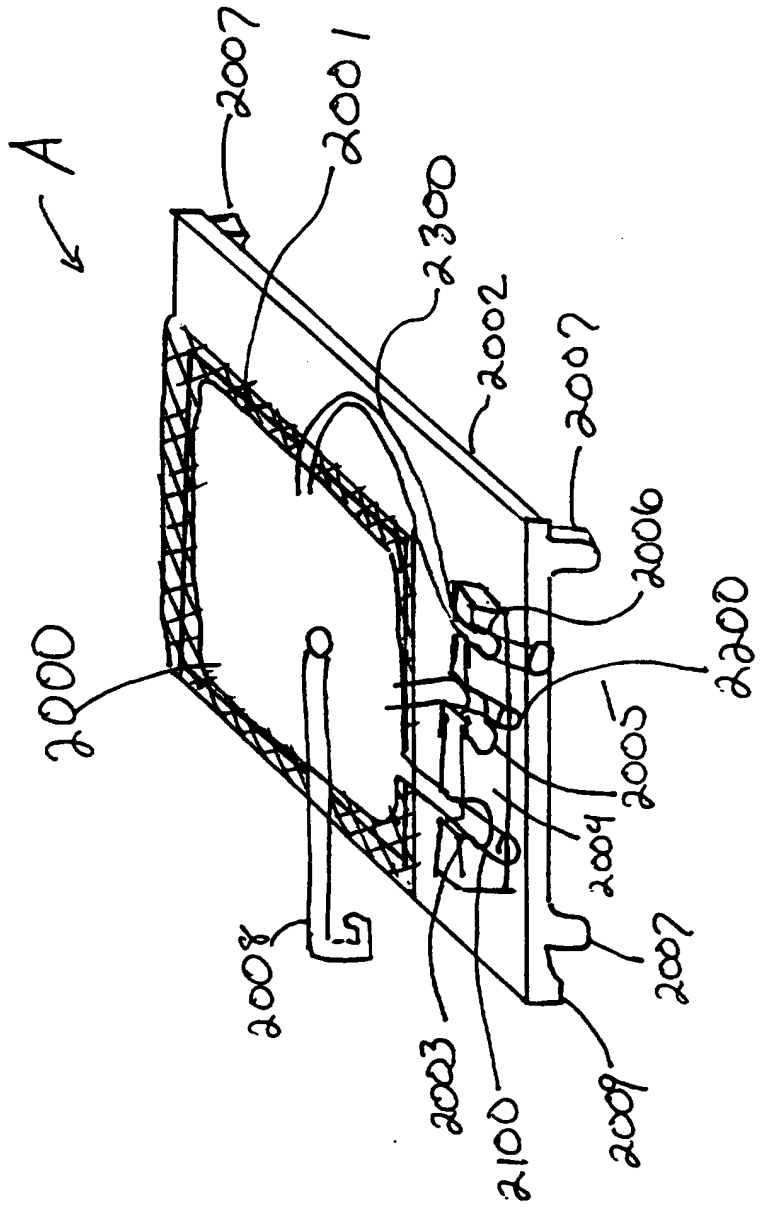

A
2007
2001
2300
2002
2007
2006
2000
2200
2005
2004
2008
2007
2003
2100
2009

FIG 17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/04205

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A01N 1/02
US CL :435/1.2, 284.1
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/1.1, 1.2, 1.3, 284.1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ------ Y	US 5,051,352 A (MARTINDALE ET AL.) 24 September 1991 (24.09.91), see entire document.	1-3, 5, 8, 10, 12, 13, 18, 20-22 ---------- 4, 6, 7, 9, 11, 14-17, 19
Y	US 4,231,354 A (KURTZ ET AL.) 04 November 1980 (04.11.90), see entire document.	4, 11, 17
Y	US 3,738,914 A (THORNE ET AL.) 12 June 1973 (12.06.73), see entire document.	7, 19
Y	EP 0,376,763 A (MCKELVEY ET AL.) 04 July 1990 (04.07.90), see entire document.	14, 15

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 10 MAY 1996	Date of mailing of the international search report 29 MAY 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer WILLIAM H. BEISNER Telephone No. (703) 308-0651

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/04205

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Database WPIDS on STN, Derwent Publications Ltd., WPIDS No. 90-358177, JP 02-258701 A (OLYMPUS OPTICAL CO LTD) 19 October 1990 (19.10.90), abstract.	16, 19
A	US 4,666,425 A (FLEMING) 19 May 1987 (19.05.87), see entire document.	1-22
A	US 5,141,847 A (SUGIMACHI ET AL.) 25 August 1992 (25.08.92), see entire document.	1-22
A	US 5,338,662 A (SADRI) 16 August 1994 (16.08.94), see entire document.	1-22
A, P	US 5,472,876 A (FAHY) 05 December 1995 (05.12.95), see entire document.	1-22
A	SCHOLZ et al. Organ Preservation Machine OKM 82. medizintechnik. 1983, Vol.23, No. 1, pages 2-5.	1-22
A	PACINI et al. Excellent Performance of the Isolated Rabbit Kidney Perfused with Platelet and Leukocyte-Poor Blood. Boll. Soc. Ital. Biol. Spec. 1980, Vol. 56, pages 2497-2503.	1-22
A	RIJKMANS et al. Six-Day Canine Kidney Preservation. Transplantation. 1984, Vol. 37, No. 2, pages 130-134.	1-22
A	PACINI et al. An Analysis of the Optimal Conditions for Perfusing an Isolated Rabbit Kidney with Homologous Blood. Renal Physiol. 1983, Vol. 6, pages 72-79.	1-22
A	US 5,285,657 A (BACCHI ET AL.) 15 February 1994 (15.02.94), see entire document.	1-22